IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Goldenberg
Serial No.: 10/002,211
Filed: December 5, 2001
Title: METHOD OF TREATING IMMUNE DISEASE USING B-CELL ANTIBODIES
Group Art Unit: 1644
Examiner: Chun Dahle
Attorney Docket No.: IMMU-0003US1
Confirmation No.: 5605

EFS-WEB

BRIEF ON APPEAL UNDER 37 CFR §41.37

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

This appeal brief is being filed in accordance with the provisions of 37 C.F.R. § 41.37. The fee of $270 under Rule 17(c) for filing of this brief is addressed in the EFS-Web generated transmittal. By way of separate paper, applicant requests an extension of time for this case, so that the brief is due on November 25, 2010, and that fee also is addressed in the EFS-Web generated transmittal. If any fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to deposit account 18-2056. If any further extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136(a) and authorizes payment of any such extensions fees to Deposit Account No. 05-0225.

I. REAL PARTY IN INTEREST

The real party in interest in this application is Immunomedics, Inc., as evidenced by an assignment filed in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to appellant, the appellant's legal representative, or the assignee which are related to, will directly affect or be directly affected by, or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

The following is the status of all pending claims and an identification of those claims that are being appealed:

Pending claims: 78-86, 93-108, 114 and 116[1]

Canceled claims: 1-77, 87-107, 109-113 and 115

Rejected claims: 78-86, 108, 114 and 116

Appealed claims: 78-86, 108, 114 and 116

IV. STATUS OF AMENDMENTS

An amendment was filed subsequent to final rejection on October 8, 2010. An Advisory Action dated October 29, 2010 indicates that this amendment was entered for purpose of appeal. All amendments have been entered and form part of the record for appeal.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Two independent claims are pending. Support for these claims is identified in brackets.

Claim 78 recites a method of ablating normal cells in a subject [page 3, lines 27-29 – "Another object of the invention is to provide methods and agents for ablating normal cells and tissues as part of a therapeutic intervention"], comprising parenterally administering to a subject [abstract – "The method comprises parenterally injecting a mammalian subject, at a locus and by a route providing access to said tissue or organ, with an composition comprising

[1] It is noted that the Advisory Action correctly indicates the cancellation of claims 109-113 and 115, but incorrectly lists the pending claims as 78-86, 108, 114 and 116. The "93-" before claim 108 is missing.

antibody/fragment which specifically binds to targeted organ, tissue or cell"] a therapeutically effective amount of a sterile injectable composition comprising a B-cell antibody or fragment thereof, which specifically binds to a B-cell [page 4, lines 23-25 -- "The method comprises parenterally injecting a mammalian subject, at a locus and by a route providing access to said tissue or organ, with a pharmaceutically effective amount" and page 12, lines 4-16 – "Antibodies and fragments useful in the methods of the present invention include those against antigens associated or produced by normal organs…Specific examples include antibodies and fragments against…B-cells"], in a pharmaceutically acceptable injection vehicle [page 23, line 8 -- "sterile conventional injection vehicles"], thereby to ablate the normal cells [page 3, lines 27-29, *supra*].

Claim 104 recites a method of treating an immune disease in a subject [page 7, lines 5-7 -- "ablation of certain normal organs and tissues for other therapeutic purposes, such as the spleen in patients with immune disease"], comprising parenterally administering to a subject that has been diagnosed with an immune disease a therapeutically effective amount of a sterile injectable composition consisting of a B-cell antibody or fragment thereof, which specifically binds to a B-cell, [page 4, lines 23-25 -- "The method comprises parenterally injecting a mammalian subject, at a locus and by a route providing access to said tissue or organ, with a pharmaceutically effective amount" and page 12, lines 4-16 – "Antibodies and fragments useful in the methods of the present invention include those against antigens associated or produced by normal organs…Specific examples include antibodies and fragments against…B-cells"], in a pharmaceutically acceptable injection vehicle [page 23, line 8 -- "sterile conventional injection vehicles"], whereby the immune disease is treated {page 7, lines 5-7 – *supra*].

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. The rejection of claims 78-86 and 93-108, 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.
2. The rejection of claims 93, 97-100 and 106-108 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.
3. The rejection of claims 102 and 105 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.
4. The rejection of claims 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

5. The rejection of claims 78-86, 93-108, 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the enablement requirement.

6. The rejection of claims 78, 81-86, 102-105, 114 and 116 under 35 USC 102(b) as being anticipated by Meyer *et al.* (US Patent 4,861,579).

7. The rejection of claims 78, 79, 81, 93, 102-107, 114 and 116 under 35 USC 102(b) as being anticipated by Bussel *et al.* (*Blood* 1988 72;1:121-127) as evidenced by de Grandamont *et al.* (*Blood* 2003 101;8:3065-3073).

8. The rejection of claims 78, 80, 93, 95-101, 107 and 108 under 35 USC 103(a) as being unpatentable over Meyer *et al.* (US Patent 4,861,579) in view of Sivam *et al.* (US Patent 5,116,944).

9. The rejection of claims 78 and 94 under 35 USC 103(a) as being unpatentable over Meyer *et al.* (US Patent 4,861,579) in view of Fishwild *et al.* (*Nature Biotech.* 1996, 14:845-851).

VII. ARGUMENT

A. The rejection of claims 78-86, 93-108, 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

1. <u>The examiner's rejection</u>

Claims 78-86, 93-108, 114 and 116 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, based on the terminology defining the active ingredient, currently defined as "a B-cell antibody or fragment thereof, which specifically binds to a B-cell." The examiner asserts that the genus of "B-cell antibody is extremely large." In this regard, she urges that the term "is not limited to those that bind B-cell surface proteins such as CD20; rather, B-cell antibody includes any antibody that binds proteins on surface of B-cells as well as those intracellular proteins."

2. <u>Summary of prosecution</u>

This written description rejection was discussed during an interview on May 17, 2007, with Examiners Gambel and Crowder. Applicant understood the result of that interview to be that the rejection for lack of written description would be obviated by amending the claims to recite "B-cell antibodies" instead of "antibody or antibody fragment specific to a marker

associated with a B cell." Indeed, the Interview Summary for that meeting states that "Applicant will consider filing a RCE and to amend the claims to recite antibodies to B cells rather than 'a marker associated with a B cell' *to obviate WD rejection of record*" (emphasis added).

Applicant then filed an RCE and amended the main claim to recite "a B-cell antibody or fragment thereof." The amendment limited the claim to antibodies that bind B-cell surface proteins. This addressed the examiner's interpretation that the unamended claims covered an extremely large group of antibodies that includes all proteins which comprise the cell surface as well as proteins that are made intracellularly, some of which become extracellular proteins secreted by the cell. The subset of B-cell antibodies is relatively circumscribed in its scope, and fully in agreement with the teaching by applicant that antibodies that bind specifically to B cells can be used to ablate normal cells and/or treat an immune disease.

However, the examiner maintained the rejection in the next action. Another interview was held on November 28, 2007, to discuss the issue further with the examiner and her supervisor, Mr. Haddad. At that interview, applicant was "encouraged to submit relevant evidence to rebut the rejection of record" – See interview summary. Applicant requested a further interview, to include a QAS in discussing the rejections. Prior to this interview, which occurred on December, 6, 2007, applicant submitted several declarations under 37 CFR 1.132, providing the "relevant evidence" suggested in the Interview Summary dated May 17, 2007. These declarations are appended in the Evidence Appendix, including all documents referenced therein.

The interview with Examiners Dahle, Gambel, Haddad and Brumback (QAS) occurred on December 6, 2007. At this meeting, Examiner Brumback felt that applicant's case would be strengthened greatly if applicant could identify B-cell antibodies available at the time of applicant's invention in 1992 which subsequently had been demonstrated to be effective in the treatment of autoimmune diseases.

In the next response, applicant provided such evidence, and also discussed in detail the import of the Rule 1.132 declarations that had been made of record in the case in advance of the December 6th interview. Applicant will now explain why the present specification includes a written description of a B-cell antibody or fragment thereof, and more particularly one which specifically binds to a B-cell (language added on October 15, 2009).

3. <u>The declaration of Kenneth Foon establishes that the term "B-cell antibody" is well understood by those of skill in the art to identify a well-defined group of antibodies</u>

The appropriate person to inform the decision of whether applicant was in possession of the claimed invention at the time of filing is a person of skill in the art, particularly one who was skilled in the art at the time of filing of the application. In order to provide evidence on this point, applicant submitted a declaration under Rule 1.132 by Dr. Kenneth Foon. Dr. Foon is an expert in the field of B-cell antibodies. In fact, during the meeting on December 3, 2007, Examiner Gambel noted that he had often, in the course of examining various cases, cited Dr. Foon's seminal review on B-cell antibodies which is referenced in Dr. Foon's declaration.

Dr. Foon's declaration addresses the examiner's concern "about whether one skilled in the art would understand that the inventor 'had possession' of the invention of using B cell antibodies to treat immune diseases." In this regard, Dr. Foon specifically addresses whether the disclosure must include evidence of "relevant identifying characteristics" and/or "a disclosed correlation between function and structure" for B-cell antibodies in order to sufficiently describe the invention to a skilled artisan. Dr. Foon notes that the examiner says that the method depends upon "finding 'B-cell antibody'" and that "without such an antibody, the skilled artisan cannot practice the claims method of treating an immune disease," and that the claims call for B-cell antibodies or fragments generally and, in her words, "[lack] a common structure essential for the function (*e.g.* antigen specificity) and the claims do not require any particular structure basis or testable function be share by the instant 'B-cell antibody or fragment thereof.'"

Dr. Foon explains why the examiner's concerns are not well-founded scientifically. First, he notes that the Cluster of Differentiation (CD) is a protocol used for the identification and investigation of cell surface molecules present on leukocytes. CD molecules act in various ways, often acting as receptors or ligands (the molecule that activates a receptor) important to a cell. Binding to the CD antigen generally initiates a signal cascade that alters the behavior of the cell. The CD nomenclature was proposed and established at the 1st International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA), which was held in 1982. The system was intended for the classification of the many monoclonal antibodies generated by different laboratories around the world against antigens/epitopes on the surface molecules of leukocytes. A proposed surface molecule is only assigned a CD number once two specific monoclonal antibodies are show to bind to the molecule. If a molecule has not been well characterized, or has only one monoclonal antibody, it is usually given the provisional indicator

"w." Attached to Dr. Foon's declaration were listings of the antigens from both the 4th and the 5th International Workshops. Since each CD represents at least two monoclonal antibodies, the attached lists show that there were many monoclonal antibodies to B cells that were known well before 1992.

Dr. Foon notes that one of the first B-cell antigens to be studied in depth was B1. In 1980, Stashenko *et al.* described and characterized a monoclonal antibody specific to this antigen. *J. Immunology*, 125(4)1678-1685 (1980). The anti-B1 antibody was studied further and the following year this group reported that all tumor cells from patients with lymphomas or chronic lymphocytic leukemias, bearing either monoclonal K or light chain, express the B1 antigen. Nadler *et al.*, *J. Clin. Invest.* 67:134-140 (1981). In 1987, Liu *et al.* described a chimeric anti-CD20 antibody, 2H7, which recognized CD20 that is expressed in normal as well as malignant B cells. *J Immunol.* 15(10):3521-6 (1987). Other scientists in the late 1980s and early 1990s were using B-cell antibodies to study the B-cell lineage of B-cell cancers, particularly (Non-Hodgkin's Lymphoma (NHL). For example, Schmid *et al.* and Shimoyama *et al.* were assessing the expression of B-cell antigens by B-cell malignancies (*Am. J. Pathology*, 139(4): 701-707 (1991) and *Japanese J. Clin. Oncol.* 13(3): 447-488 (1983), respectively).

Dr. Foon also attests that not only were researchers describing anti-B-cell antibodies to characterize the B-cell lineage of B-cell malignancies, but in the late 1980s and early 1990s they also began to describe the use of B-cell antibodies for immunotherapy of B-cell malignancies. Press *et al.* used an anti-CD20 antibody, 1F5, to treat patients with refractory malignant B-cell lymphomas. *Blood*, 96(2):584-591 (1987). While the effect was transient, the study showed that the binding of B-cell antibodies affected function of the targeted cells. This same group reported on the use of MB1, an anti-CD37 antibody, to successfully treat a small cohort of patients with NHL. Press *et al.*, *J Clin Oncol.* 7(8):1027-38 (1989).

Dr. Foon attests that a large number of B-cell antibodies directed to different B-cell antigens had already been developed by 1992, and that the genus of B-cell antibodies possesses a commonality of function. The function shared by members of the genus is the ability to bind to a B-cell antigen. Dr. Foon was the lead author on a paper published in 1987 entitled "Immunologic Classification of Leukemia and Lymphoma" (Foon and Todd, *Blood*, 68(1):1-31 (1987)). Table 1 of that article lists 30 monoclonal antibodies reactive with human B lymphocytes. Many of these were commercially available from companies such a Coulter

Immunology and Ortho System, Inc. Thus, the disclosure of "B-cell antibodies" described to a person of skill in the art a large number of different antibodies, and not just the LL2 antibody that is mentioned in the above-captioned application. Many of these antibodies were freely available to those of skill in the art.

Dr. Foon's article also discloses that B-cell antibodies are useful in monoclonal antibody therapy of B-cell cancers. The article cites one study in which patients were treated with the BA-1, BA-2 and BA-3 monoclonal antibodies to B cells, and another in which patients were treated with anti-B1 antibody. These studies both showed that the binding of B-cell antibodies to cancerous B cells affects disease progression. Thus, B-cell antibodies have been demonstrated to possess a commonality of function both in terms of their ability to specifically bind to B cells and also in the ability to affect disease progression as a result of that binding. In the conclusion section of his paper Dr. Foon accurately predicted that B-cell antibodies would be found to be useful in the therapy of leukemias and lymphomas.

Patent applications for specific B-cell antibodies already were being filed in the late 1980s and early 1990s. For example, Robinson *et al.* filed an application in 1987 that was directed to the use of an anti-CD20 antibody in treating B-cell malignancies. This was published in 1988 as WO8804936 and gave rise to a number of US patents, including US5721108, US6204023, US6652852, US6893625 and others. These patents claim the 2H7 antibody that recognizes the BP35 anti-CD20 antigen. Ledbetter *et al.* filed an application in 1986 that disclosed the antibody G28-5. The antibody was used to define the B-cell receptor Bp50, and claims to the BP50 antigen issued in US 5,247,069. Each of these applicants deposited a hybridoma which secreted their claimed antibody.

The foregoing articles and patents establish that a large number of B-cell antibodies had been described and were commercially and/or publicly available prior to 1992. Therefore, Dr. Foon concludes that a skilled artisan, reading the disclosure in the present case that:

- "ablation of certain normal organs and tissues for other therapeutic purposes, such as the spleen in patients with immune disease or lymphomas, the bone marrow in patients requiring bone marrow transplantation, or normal cell types involved in pathological processes, such as certain T-lymphocytes in particular immune diseases" (page 7, lines 5-10)

- "Another therapeutic application for such organ- and tissue-targeting antibodies conjugated with a toxic agent is for the ablation of certain normal cells and tissues as part of another therapeutic strategy, such as in bone marrow ablation with antibodies against bone marrow cells of particular stages of development and differentiation, and in the cytotoxic ablation of the spleen in patients with lymphoma or certain immune diseases, such as immune thrombocytopenic purpura, etc." (page 9, lines 2-10)

- "Specific examples include antibodies and fragments against bone marrow cells, particularly hematopoietic progenitor cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, B-cells, T-cells, Null cells, vascular endothelial cells, bile duct cells, gall bladder cells, prostate cells, hormone receptors such as of FSH, LH, TSH, growth factor receptors, such as of epidermal growth factor, urinary bladder cells, and vas deferens cells" (page 12, lines 12-20) and

- "Antibodies that target the spleen well include the LL2 (also known as EPB-2) monoclonal antibody, disclosed in Pawlak-Byczkowska, cancer Research, 49:4568-4577 (1989), which is directed against normal and malignant B cells, and which can be used for treating normal spleen cells in patients with immune diseases, lymphoma, and other diseases" (page 12, lines 30-35)

"would understand that the applicant was in possession of a method of using B-cell antibodies generally to treat immune diseases, and not just the LL2 B-cell antibody specifically. The skilled artisan would understand that the contribution to the art was the teaching that B-cell antibodies generally could be used to treat immune diseases. These B-cell antibodies have a commonality of function, in that they all bind to B-cell surface antigens. In another context, that of B-cell cancers, this commonality of function has been found to correlate to an ability to affect disease progression as a result of that binding (I have discussed this in paragraph 7 above). This binding function is one that is testable, as I described in paragraph 3 above, and the skilled

artisan would not need to know the structure of particular B-cell antibodies in order to be apprised of, and to practice, the full scope of this invention." This conclusion is repeated in the concluding paragraph of a second Rule 1.132 declaration submitted by applicant, that of Dr. Dörner.

Dr. Foon and Dr. Dörner directly counter the examiner allegations that "Given that there exist a large amount of B cell proteins that can be used to make B-cell antibodies, the structure of the species within the claimed genus would be expected to vary unpredictably from the structure of the single, described LL2 antibody." As a highly regarded expert in this field, Dr. Foon's statements with respect to how a skilled artisan would understand the term B-cell antibody and fragments thereof are highly probative on the issues of written description and possession of the invention with respect to this terminology in applicant's claims. It is precisely the type of evidence mentioned by Examiner's Haddad and Dahle in the Interview Summary of November 28, 2007.

4. <u>B-cell antibodies were readily available circa 1992 and earlier</u>

There were many B-cell antigens antibodies that were publicly available prior to the 1992 priority date of the present claims. Various murine B-cell antibodies were taught for treating NHL patients as early as the late 1980's. See, for example, Press *et al.*, "Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas," *Blood*, 1987 Feb;69(2):584-91; Press *et al.*, "Treatment of refractory non-Hodgkin's lymphoma with radiolabeled MB-1 (anti-CD37) antibody," *J Clin Oncol.*, 1989 Aug;7(8):1027-38; and Nadler *et al.*, "Anti-B1 monoclonal antibody and complement treatment in autologous bone-marrow transplantation for relapsed B-cell non-Hodgkin's lymphoma," *Lancet*, 1984 Aug 25;2(8400):427-31. Other B cell antibodies were known. For example, Mason *et al.* disclose anti-CD22 antibodies To15 and 4KB128, anti-CD19 antibody B4 and anti-CD21 antibody B2 (*Blood*, 1987 Mar;69(3):836-40). The Dana Farber group developed the B1 Mab against CD20 in the 80's and licensed it to Coulter for flow cytometry, and B-cell antibodies against other antigens, such as CD19, also were routinely used to identify B-lymphocytes by immunocytology of blood and tissue specimens. These antibodies were commercially available from various companies, including Becton-Dickinson and others. Moreover, Anderson *et al.* demonstrates that the making of antibodies to B cell antigens was within the level of skill in the art circa 1984, the date of his article (Anderson *et al.*, "Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell

differentiation," *Blood*, 1984 Jun;63(6):1424-33 - "The techniques of immunization, somatic cell hybridization, and selection of hybridomas that were used to produce the monoclonal antibodies directed at the Ia, B4, CALLA, Bi, B2, sIg, Ti, Tb, PC- I, and PCA-1 antigens have been previously described"). Copies or abstracts of these articles first were submitted May 24, 2007, and are appended in the Evidence Appendix.

Applicant's contribution, as recited in the present claims, is the teaching that B-cell antibodies are useful in the treatment of immune diseases. Armed with this information, the skilled artisan could readily practice this teaching by using commercially available B-cell antibodies or by making other B-cell antibodies according to established methodologies, as supported by Anderson *et al.*

5. <u>Evidence that B-cell antibodies which existed in 1992 are effective in treating autoimmune disease</u>

As noted above, Examiner Brumback stated at the interview that evidence that B-cell antibodies in existence at the time of filing of the present application had subsequently been shown to be effective in the treatment of an autoimmune disease would be highly informative with respect to the Section 112 issue of record. Applicant therefore researched the literature in order to place such evidence into the record.

Several of the B-cell antibodies known in 1992 have been tested for efficacy in treating autoimmune diseases. These include epratuzumab. Epratuzumab is a humanized version of the anti-CD22 antibody LL2 which is mentioned in applicant's specification. Dörner *et al.*, *Arthritis Res Ther.* 2006; 8(3): R74 reports an initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus. Steinfeld *et al.* report that "recent studies have demonstrated the efficacy and safety of epratuzumab in several autoimmune diseases, including systemic lupus erythematosus and primary Sjögren's syndrome." *Expert Opin Biol Ther.* 2006 Sep;6(9):943-9. Copies of both of these articles were submitted during prosecution on December 26, 2007, and are appended in the Evidence Appendix.

Trubion has a Fab-based product made from 1F5, another B-cell antibody that was known in 1992, as evidenced by Dr. Foon's declaration. This antibody is being tested in rheumatoid arthritis by Wyeth, who licensed the antibody. A press release from Trubion is appended (originally submitted December 26, 2007).

A humanized version of the anti-CD20 antibody 2H7 also has been shown to be effective in treating autoimmune diseases. 2H7 is another antibody referenced in Dr. Foon's declaration. This antibody, named ocrelizumab, is being tested in the treatment of rheumatoid arthritis. Genentech has announced that ocrelizumab met its primary endpoint of safety in all doses studied and also met its secondary endpoint of clinical activity at all dose levels studied in a Phase I/II ACTION study of rheumatoid arthritis.

Thus humanized versions of three antibodies known in 1992 have been shown to be effective in the treatment of various autoimmune diseases. This further supports the patentability of the present claims which are directed to the treatment of immune diseases with B-cell antibodies.

Based on the foregoing, it is clear that the present specification provides a written description of the invention recited in the present claims, *i.e.*, the treatment of immune diseases with B-cell antibodies, and conveys to one skilled in the art that applicant had possession of that invention at the time of filing.

B. The rejection of claims 93, 97-100 and 106-108 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

1. <u>The examiner's rejection</u>

Claims 93, 97-100 and 106-108 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, based on the terminology "chimeric or hybrid antibody which binds multiple epitopes or antigens." The examiner urges that these claims recite a genus "a B-cell antibody or fragment thereof ... wherein the antibody or fragment thereof is an antibody having multiple epitope or multiple specificity" as part of the invention without providing a physical structure or testable functional activity for the antibody or fragment thereof. The genus of the "a B-cell antibody or fragment thereof ... wherein the antibody or fragment thereof is an antibody having multiple epitope or multiple specificity" are therefore extremely large. Applicant has disclosed a genus of antibody with dual or multiple antigen or epitope specificity without setting forth any species (*e.g.* actual epitopes or antigens). Thus Applicant has not disclosed any species of the "a B-cell antibody or fragment thereof ... wherein the antibody or fragment thereof is an antibody having multiple epitope or multiple specificity". The claimed "a B-cell antibody or fragment thereof ... wherein the antibody or fragment thereof

is an antibody having multiple epitope or multiple specificity" lack a common structure essential for their function and the claims do not require any particular structure basis or testable functions be shared by the instant antibody."

2. The specification provides a written description of the term "chimeric or hybrid antibody which binds multiple epitopes or antigens" which evidences possession by applicant of the invention as claimed in claims 93, 97-100 and 106-108

As attested to by Dr. Foon, and described in detail above, "B-cell antibodies have a commonality of function, in that they all bind to B-cell surface antigens. In another context, that of B-cell cancers, this commonality of function has been found to correlate to an ability to affect disease progression as a result of that binding (I have discussed this in paragraph 7 above). This binding function is one that is testable, as I described in paragraph 3 above, and the skilled artisan would not need to know the structure of particular B-cell antibodies in order to be apprised of, and to practice, the full scope of this invention." This conclusion is repeated in the concluding paragraph of a second Rule 1.132 declaration submitted by applicant, that of Dr. Dörner. This argument applies equally to chimeric or hybrid antibodies which bind to multiple epitopes or antigens, and a skilled artisan would understand applicant to be in possession of antibodies with multiple binding capabilities.

Applicant's contribution, as recited in the present claims, is the teaching that B-cell antibodies are useful in the treatment of immune diseases. This also applies to chimeric or hybrid B-cell antibodies which bind to multiple epitopes or antigens. The skilled artisan could readily practice this teaching by making such hybrid and chimeric B-cell antibodies according to established methodologies.

Based on the foregoing, it is clear that the present specification provides a written description of the invention recited in the present claims, *i.e.*, the treatment of immune diseases with a chimeric or hybrid B-cell antibody which binds multiple epitopes or antigens, and conveys to one skilled in the art that applicant had possession of that invention at the time of filing.

C. The rejection of claims 102 and 105 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

1. The examiner's rejection

Claims 102 and 105 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, based on the terminology "B-cell immune disease." The examiner urges that:

> The specification only discloses "immune disease" (see page 12 of the specification or see below)
>
> > Antibodies that target the spleen well include the LL2 (also known as EPB-2) monoclonal antibody, disclosed in Pawlak-Byczkowska, cancer Research, 49:4568-4577 (1989), which is directed against normal and malignant B-cells, and which can be used for treating normal spleen cells in patients **with immune diseases, lymphoma, and other diseases**.
>
> The instant claims now recite "B-cell immune disease" which is not clearly disclosed in the specification. Therefore, the claims represent a departure from the specification and claims originally filed. Applicant's reliance on generic disclosure (an immune disease) do not provide sufficient direction and guidance to the features currently claimed. (emphasis in original)

2. The specification provides a written description of the term "B-cell immune disease" which evidences possession by applicant of the invention as claimed in claims 102 and 105

Claims 102 and 105 were proposed in response to a suggestion by the examiner. The claims are supported by the specification at page 12, lines 30-35. Page 12 of the specification discloses LL2 targets B cells and is useful to treat immune disease. Thus, the term "B-cell immune disease" is supported by the instant specification.

In order to address the examiner's position with respect to the term "immune disease," applicant submitted the declaration of Dr. Dörner, an expert in the field of autoimmune diseases. Portions of the declaration also are probative on the separate issue of "B-cell" immune disease as recited in claims 102 and 105.

Dr. Dörner is extremely well qualified to opine on the issue of the scope of the term immune disease circa 1992, and he attests, as an immunologist and rheumatologist, that in the context of the entire disclosure of the present application, he finds the term "immune disease" is

used in conjunction with a discussion of the use of a B-cell antibody and also in conjunction with a disclosure of the ablation of normal spleen cells and a disclosure of “certain immune diseases, such as immune thrombocytopenic purpura,” and he immediately recognized that the reference in the disclosure of “antibodies that target the spleen,” is a reference to a targeting of immune cells that reside in the spleen. He explains that B-cell hematologic abnormalities are a consequence of immune diseases in which the immune system is positively regulated, and immune thrombocytopenic purpura (ITP) is an example of such an immune disease. In particular, he points out that B cells differentiating into plasma cells are known to make antibodies, including the autoantibodies considered to be responsible for destroying platelets in ITP. Based on the foregoing, it is clear that Dr. Dörner immediately made the connection between the term “immune disease” and “B-cell immune disease.” Accordingly, the specification clearly shows that applicant possessed methods of treating B-cell immune diseases, and the specification conveys that to the skilled artisan, as evidenced by Dr. Dörner’s statements.

D. The rejection of claims 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

1. The examiner’s rejection

Claims 114 and 116 are rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement. This rejection is based on the term "a marker associated with a B cell." The Examiner urges that this term is not supported by the disclosure as filed, stating that the specification only discloses LL2 monoclonal antibody that target the spleen cell; the instant claims now recite any antibody specific to a marker associated with a B cell, which were not clearly disclosed in the specification.”

2. The specification provides a written description of the term “marker associated with a B cell” which evidences possession by applicant of the invention as claimed in claims 114 and 116

The specification describes that a “marker” is an entity to which an antibody or antibody fragment binds. Thus, page 15, lines 8-9 references that “the antibody is an antibody or antibody fragment which specifically binds a ***marker*** produced by or associated with said cell or tissue.” Page 5, lines 19 and 20 describe “an antibody or antibody fragment specific to a marker associated with or produced by bone marrow cells,” and page 12, lines 12-16 describes “antibodies and fragments against bone marrow cells, particularly hematopoietic progenitor

cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, B-cells…" Page 12, lines 30-33 discloses that "Antibodies that target the spleen well include the LL2 (also known as EPB-2) monoclonal antibody, disclosed in Pawlak-Byczkowska, *Cancer Research*, 49:4568-4577 (1989), which is directed against normal and malignant B-cells." Accordingly, "an antibody or antibody fragment specific to a marker associated with a B cell" were clearly disclosed in the parent specification and the present claims are not a "departure" from the specification and claims as originally filed.

A skilled artisan can readily determine whether an antibody or antibody fragment is specific for a marker associated with a B cell. There exist cultured cell lines that express various B cell antigens. A skilled artisan readily can assess whether an antibody binds to the B cell antigen on these cell lines, and therefore there is a testable functional activity associated with the term "specific for a marker associated with a B cell." This was addressed in Dr. Foon's declaration, in which he stated that "[B-cell] binding function is one that is testable, as I described in paragraph 3 above."

Stein *et al.*, *Cancer Immunol Immunother*, 1993, 37(5):293-8 (abstract appended, first submitted December 18, 2006), describes studies in which the specificity of LL2 was determined by binding studies with cultured cell lines, Nalm-6 and Molt-4. The binding profile of LL2 on these cell lines was consistent with anti-CD22, but not anti-CD21. Sequential immunoprecipitation and cross-blocking studies with anti-CD22 monoclonal antibodies recognizing established CD22 epitopes were performed to confirm that LL2 reacts with CD22 and to determine which epitope LL2 recognizes. Antibodies to other B cell markers can similarly be "tested" with cultured cell lines that express other of the B cell markers, such as CD19 and CD20, and thus antibodies to any of the B cell markers have a testable functional activity and hence are fully described.

Based on the foregoing, it is clear that the present specification provides a written description of the invention recited in the present claims, *i.e.*, the treatment of immune diseases with a chimeric or hybrid B-cell antibody which binds multiple epitopes or antigens, and conveys to one skilled in the art that applicant had possession of that invention at the time of filing.

E. The rejection of claims 78-86, 93-108, 114 and 116 under 35 USC 112, first paragraph, as failing to comply with the enablement requirement.

1. The examiner's rejection

Claims 78-86, 93-108, 114 and 116 are rejected under 35 USC 112, first paragraph, as failing to comply with the enablement requirement. The examiner urges that "a B-cell antibody .... which specifically binds to a B-cell" does not limit the claims to only antibody exclusively binds B cells. In addition, the specification does not provide sufficient guidance and direction as to how to make the claimed B-cell antibody. The specification discloses only generic methods of making antibody by using whole cell or cell extract as antigen… One of skill in the art would not be able to make the claimed B-cell antibody that specifically binds only B-cells and to use it in the claimed method of ablating normal cells and/or a method of treating an immune disease by administering the antibody produced under guidance of the instant specification."

2. The specification enables the skilled artisan to make and use B-cell antibodies to treat an immune disease as recited in claims 78-86, 93-108, 114 and 116

The examiner raised this rejection following comments made by applicant with respect to Meyer. In addressing a novelty rejection based on Meyer, applicant said "Although Meyer's results with Lym-1 and Lym-2 show some B-cell activity, these are HLA-DR antibodies. The literature clearly shows that this class of antibodies reacts with more than just B-cells, and even with some solid tumors," and "The only species of antibodies disclosed by Meyer *et al.* are not B-cell antibodies (Meyer's characterization being inaccurate in this regard) and they are expressed only at very low levels on normal cells."

A skilled artisan does not understand the term "B-cell antibody" in applicant's claims to include HLA-DR antibodies such as Lym-1 and Lym-2. A skilled artisan would not understand the Lym-1 or Lym-2 antibodies to be B-cell antibodies and would not select them for use in the present invention. The fact that Meyer's antibodies have some minor B-cell activity does not make them "B-cell antibodies." Significantly, although first described in the literature in 1987,[2] they are not listed among the B-cell antibodies in either of the 4th or 5th Workshops which followed.[3]

[2] Epstein *et al.* 1987, which is referenced in Meyer *et al.*
[3] The Workshops are attached to the Foon declaration.

Applicant enables the skilled artisan to use B cell antibodies which specifically bind to B-cells to ablate normal cells and treat immune disease, and was making the point that Meyer does not disclose B cell antibodies, as that term is understood by those of skill in the art and used by applicant.

F. The rejection of claims 78, 81-86, 102-105, 114 and 116 under 35 USC 102(b) as being anticipated by Meyer et al. (US Patent 4,861,579).

1. <u>The examiner's rejection</u>

Claims 78, 81-86, 102-105, 114 and 116 are rejected under Section 102(b) based on Meyer *et al.* (US 4,861,579). The examiner urges that:

> Meyer *et al.* teach a method of treating immune diseases such as infection, autoimmune disease by administering an anti-B antibody or fragment thereof (see entire document, particularly columns 1-3). Meyer *et al.* further teach that said antibody can be conjugated with therapeutic agents such as radioisotopes, toxins, cytotoxic agents (*e.g.*, see column 2).

2. <u>Meyer teaches use of Lym-1 and Lym-2 to suppress the side effects arising from treatment of autoimmune disease and not to treat an immune disease</u>

Meyer, cited in a rejection under Section 102 and as the primary reference in two rejections under Section 103, would not have suggested a method as presently claimed. Meyer relates to use of an anti-B cell antibody for suppressing the immune response generated upon administration of a therapeutic agent administered either as a naked or a conjugated antibody. Accordingly, there is no disclosure in Meyer that the anti-B-cell antibody is used to ablate normal cells, rather Meyer teaches how to combat the side effects arising from therapy using, for diagnostic or therapeutic purposes, an antibody (page 2, lines 38-42). The treatment modality may, according to Meyer also be used in connection with the use of therapeutic antibodies in the treatment of autoimmune diseases (page 3, lines 47-49). In other words, according to Meyer the side effects arising from the treatment of autoimmune diseases using antibodies may be treated using an antibody against the B-lymphocytes. Meyer does not teach a method of ablating normal cells in a subject, in which a therapeutically effective amount of a sterile injectable composition comprising a B-cell antibody or fragment thereof in a pharmaceutically acceptable injection vehicle is administered to treat an immune disease.

The examiner responded to these comments by noting that "in contrast to applicant's reliance on the preamble of the claims, it is noted that the claimed language or limitation does not appear to result in a manipulative difference in the method steps when compared to the prior art disclosure." Applicant subsequently amended claim 78 to recite *in the body of the claim* "thereby to ablate the normal cells" and amended claim 104 to recite "thereby to treat the immune disease." This addressed the statement that applicant is relying on the preamble.

3. <u>The Lym-1 and Lym-2 antibodies are not B-cell antibodies</u>

Furthermore, the Lym-1 and Lym-2 antibodies of Meyer are not considered to be B-cell antibodies. Although Meyer's results with Lym-1 and Lym-2 show some B-cell activity, these are HLA-DR antibodies. The literature clearly shows that this class of antibodies reacts with more than just B-cells, and even with some solid tumors. For example, Würflein *et al.* 1998 evaluated antibodies for their capacity to induce cell-mediate lysis of malignant B cells, and "compared killing mediated by chimeric IgG1 antibodies with that from FcyRI-directed bispecific antibodies, targeting classical HLA class II, or the Lym-1 and Lym-2 antigens. The latter two are variant forms of HLA class II, which are highly expressed on the surface of malignant B cells but which are found only at low levels in normal cells."

Würflein *et al.* further notes that "Lym-1 and Lym-2 may have the additional advantage that they bind preferentially to HLA class II in malignant human B cells compared to normal B cells and monocytes." Epstein *et al.* 1987, which is referenced in Meyer *et al.*, cites facts in agreement with the low level of expression on normal cells and with the HLA-DR specificity of these antibodies noted by Würflein *et al.* Low levels of binding to normal B cells leads to the conclusion that the antibodies are not "B-cell antibodies" and would *not* be effective for treating immune diseases.

The only species of antibodies disclosed by Meyer *et al.* are not B-cell antibodies (Meyer's characterization being inaccurate in this regard) and they are expressed only at very low levels on normal cells. This is in clear contrast to applicant's disclosed species of EPB2, which is a B-cell antibody and is well expressed on normal B cells. Unlike applicant's disclosure, Meyer's disclosure does not provide a written description that evidences possession of an invention of treating autoimmune diseases with B-cell antibodies. Copies of Würflein *et al.* and Epstein *et al.* are appended, and were first submitted December 26, 2007. Therefore, Meyer does not teach treatment of an immune disease with a B cell antibody.

G. The rejection of claims 78, 79, 81, 93, 102-107, 114 and 116 under 35 USC 102(b) as being anticipated by Bussel *et al.* (*Blood* 1988 72;1: 121-127) as evidenced by de Grandamont *et al.* (*Blood* 2003 101;8:3065-3073).

1. <u>The examiner's rejection</u>

Claims 78, 79, 81, 93 and 102-107 are rejected under Section 102(b) based on Bussel *et al.* as evidenced by Grandamont *et al.* Bussel *et al.* is cited as showing the treatment of ITP with IVIG. Grandamont *et al.* is cited as evidencing that IVIG includes antibodies reactive against antigens on B lymphocytes. In this regard, the examiner cites "the entire document" but particularly page 3065.

2. <u>Grandamont *et al.* fails to provide the evidence urged that IVIG in Bussel contains B-cell antibodies and certainly not in a therapeutically effective amount as presently claimed</u>

Upon careful review of Bussel *et al.*, applicant is unable to find any disclosure that IVIG includes antibodies against B lymphocytes. The role of the Fc region of IVIG is mentioned on page 3065, and then the potential contribution of anti-idiotype antibodies, but there is no disclosure that IVIG includes B-cell antibodies. Moreover, the model used in Grandamont was "an *in vitro* culture system that reproduces T-dependent activation of B lymphocytes through the binding of CD40 on B lymphocytes." CD40 is a receptor molecule on the cell surface of many cell types, including monocytes, dendritic cells (in the nervous system), endothelial cells (within blood vessels), and epithelial cells, in addition to mature B cells and most B-cell malignancies. Thus, Grandamont *et al.* fails to provide the evidence urged that IVIG contains B-cell antibodies, and further suggests that the effects observed for IVIG have to do with T-dependent activation of B lymphocytes by an unidentified mechanism.

Grandamont *et al.* discusses the role of the Fc region of IVIG in treatment, as mentioned on page 3065 of that document, and fails to teach that IVIG includes B cell antibodies, let alone teaching or suggesting the use of B-cell antibodies in the treatment of ITP or any other autoimmune disease. The primary mechanism of IVIG has been proposed to be the blockade of Fc-receptors, as noted in Teeling *et al.*, "Therapeutic efficacy of intravenous immunoglobulin preparations depends on the immunoglobulin G dimers: studies in experimental immune thrombocytopenia," *Blood*, 2001, Aug 15;98(4):1095-9 (copy appended – first submitted February 4, 2008):

> The clinical benefit of intravenous immunoglobulin (IVIG) preparations in the treatment of immune thrombocytopenic purpura (ITP) is supposed to be mediated by blockade of Fcγ receptor–bearing phagocytes (abstract).

and

> Low-affinity Fcγ receptors apparently were involved in the antibody dependent clearance of platelets in our model because *in vivo* blocking of FcγRII/III or the absence of these receptors in knock-out mice completely prevented the effect of antiplatelet mAb. The importance of low-affinity Fcγ receptors in the clearance of immune complexes has been shown in several human and animal studies.[6] Furthermore, infusion of mAbs against FcγRIII caused a significant, transient increase in platelet counts in a patient with ITP.[10] (page 1098, last paragraph)

Teeling sought to clarify the entity in IVIG responsible for the therapeutic effect, and discovered that it was dimers ("We demonstrated that clearance of platelets sensitized with anti-platelet mAb could only be reduced by administration of an IVIG preparation containing significant amounts of IgG dimmers" – page 1098, first paragraph of "Discussion").

Teeling goes on to discount a proposed mechanism of idiotype-anti-idiotype interaction with autoantibodies:

> An alternative mechanism for the reversal of thrombocytopenia by IVIG involves idiotype-anti-idiotype interaction with autoantibodies and anti-idiotype antibodies in the IVIG preparations.[5] This mechanism is independent of RES blockade, but it postulates the neutralization of antiplatelet antibody by IVIG. However, it was shown that Fc fragments have the same therapeutic effects as IVIG in patients with ITP.[27] Thus, anti-idiotypic effects of IVIG are not likely a major mechanism of action in ITP. In our study, binding of MWReg30 to platelets was not influenced by IVIG, nor did we find (anti-idiotypic) antibodies with binding capacity to MWReg30.

Certainly the fact that Fc-fragments have the same activity in patients as IVIG goes against an assertion of any possible antibody effect of the preparation. Even assuming, *arguendo*, that IVIG does include B-cell antibodies, it is clear that the amount is not sufficient to anticipate a claim which recites "a therapeutically effective amount" of B-cell antibody. The art clearly shows that the therapeutically active ingredient in IVIG is dimers interacting with Fcγ receptors, not B-cell antibodies. Accordingly, the rejection under Section 102(b) based on Bussel *et al.* as evidenced by Grandamont *et al.* is *prima facie* defective.

H. The rejection of claims 78, 80, 93, 95-101, 107 and 108 under 35 USC 103(a) as being unpatentable over Meyer et al. (US Patent 4,861,579) in view of Sivam et al. (US Patent 5,116,944).

1. The examiner's rejection

Claims 78, 80, 82-86, 95 and 101 are rejected under Section 103 based on Meyer *et al.* in view of Sivam *et al.* (US 5,116,944). Meyer *et al.* is discussed above. Sivam *et al.* is cited as teaching antibody fragments and antibody conjugated to a cytokine.

2. Sivam *et al.* does not overcame the failure of Meyer to teach treatment of an immune disease with a B-cell antibody

The arguments relating to Meyer in Section VII.G are incorporated here by reference. Sivam *et al.* is relied upon only as teaching antibody fragments and antibody conjugated to a cytokine. Thus, Sivam fails to overcome Meyer's failure to teach treatment of an immune disease with a B-cell antibody. No *prima facie* case of obviousness exists.

I. The rejection of claims 78 and 94 under 35 USC 103(a) as being unpatentable over Meyer et al. (US Patent 4,861,579) in view of Fishwild et al. (Nature Biotech. 1996, 14:845-851).

1. The examiner's rejection

Claims 78 and 94 are rejected under Section 103 based on Meyer *et al.* in view of Fishwild *et al.* 1996. Fishwild *et al.* is cited as teaching human monoclonal antibodies.

2. Fishwild *et al.* does not overcame the failure of Meyer to teach treatment of an immune disease with a B-cell antibody

The arguments relating to Meyer in Section VII.G are incorporated here by reference. Fishwild *et al.* is relied upon only as teaching human monoclonal antibodies. Thus, Fishwild *et al.* fails to overcome Meyer's failure to teach treatment of an immune disease with a B-cell antibody. No *prima facie* case of obviousness exists.

VIII. CONCLUSION

For these reasons, the Board is requested to reverse the decision of the examiner and pass the present case to issuance.

Respectfully submitted,
ROSSI, KIMMS & McDOWELL LLP

NOVEMBER 26, 2010
DATE

/Barbara A. McDowell/

Barbara A. McDowell
Reg. No. 31,640

20609 Gordon Park Square, Suite 150
Ashburn, VA 20147
703-726-6020 (phone)
703-726-6024 (fax)

CLAIMS APPENDIX

This appendix contains a copy of the appealed claims, with their status identifiers.

78. (Previously presented) A method of ablating normal cells in a subject, comprising parenterally administering to a subject a therapeutically effective amount of a sterile injectable composition comprising a B-cell antibody or fragment thereof, which specifically binds to a B-cell, in a pharmaceutically acceptable injection vehicle, thereby to ablate the normal cells.

79. (Previously presented) A method according to claim 78, wherein the subject has been diagnosed with immune thrombocytopenic purpura.

80. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is a Fv, single chain antibody, Fab, Fab', or $F(ab')_2$ fragment.

81. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is an intact antibody.

82. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is conjugated to a therapeutic agent.

83. (Previously presented) A method according to claim 82, wherein the therapeutic agent is a cytotoxic agent.

84. (Previously presented) A method according to claim 83, wherein the cytotoxic agent is a therapeutic radioisotope.

85. (Previously presented) A method according to claim 82, wherein the therapeutic agent is a drug.

86. (Previously presented) A method according to claim 82, wherein the therapeutic agent is a toxin.

93. (Previously presented) A method of ablating normal cells in a subject, comprising parenterally administering to a subject a therapeutically effective amount of a sterile injectable composition comprising a B-cell antibody or fragment thereof, which specifically binds to a B-cell, in a pharmaceutically acceptable injection vehicle, wherein the antibody or fragment thereof is a polyclonal, chimeric or hybrid antibody which binds multiple epitopes or antigens, thereby to ablate the normal cells.

94. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is a human monoclonal antibody.

95. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is a mouse/human chimeric monoclonal antibody.

96. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is a genetically engineered antibody.

97. (Previously presented) A method according to claim 93, wherein the antibody or antibody fragment is conjugated to a therapeutic agent.

98. (Previously presented) A method according to claim 97, wherein the antibody or antibody fragment is conjugated to a cytotoxic agent.

99. (Previously presented) A method according to claim 93, wherein the antibody or antibody fragment is conjugated to a drug.

100. (Previously presented) A method according to claim 93, wherein the antibody or antibody fragment is conjugated to a radioisotope.

101. (Previously presented) A method according to claim 78, wherein the antibody or antibody fragment is conjugated to a cytokine.

102. (Previously presented) A method of treating an immune disease in a subject according to claim 78, wherein said immune disease is a B-cell immune disease.

103. (Previously presented) A method of treating an immune disease in a subject according to claim 78, wherein said antibody or antibody fragment is a B-cell antibody.

104. (Previously presented) A method of treating an immune disease in a subject, comprising parenterally administering to a subject that has been diagnosed with an immune disease a therapeutically effective amount of a sterile injectable composition consisting of a B-cell antibody or fragment thereof, which specifically binds to a B-cell, in a pharmaceutically acceptable injection vehicle, whereby the immune disease is treated.

105. (Previously presented) A method of treating an immune disease in a subject according to claim 104, wherein said immune disease is a B-cell immune disease.

106. (Previously presented) A method according to claim 93, wherein the subject has been diagnosed with immune thrombocytopenic purpura.

107. (Previously presented) A method according to claim 93, wherein the antibody or antibody fragment is an intact antibody.

108. (Previously presented) A method according to claim 93, wherein the antibody or antibody fragment is conjugated to a therapeutic agent.

114. (Previously presented) A method according to claim 78, wherein the antibody is specific to a marker associated with a B cell.

116. (Previously presented) A method according to claim 104, wherein the antibody is specific to a marker associated with a B cell.

RELATED PROCEEDINGS APPENDIX

There are no related appeals or interferences known to appellant the appellant's legal representative, or assignee which are related to, will directly affect or be directly affected by, or have a bearing on the Board's decision in the pending appeal.

EVIDENCE APPENDIX

These documents were entered into the record on December 18, 2006.

Goldenberg, et al., "Targeting, dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labeled LL2 monoclonal antibody" *J Clin Oncol.* 1991 Apr;9(4):548-64.

Sixteen patients with non-Hodgkin's lymphoma were infused with 6.2 to 58.2 mCi (0.2 to 3.9 mg) doses of radioactive iodine (131I)-labeled LL2 immunoglobulin G (IgG) or F(ab')2, in order to study antibody distribution, pharmacokinetics, dosimetry, toxicity, tumor targeting, and therapy. LL2 is a murine IgG2a monoclonal antibody (MAb) reactive with B cells and non-Hodgkin's B-cell lymphoma. In a series of five assessable therapy patients, doses as small as 30 mCi 131I-LL2 IgG or F(ab')2 resulted in tumor responses (two partial remissions, two mixed and minor responses, and one no response), while one patient receiving diagnostic doses as low as 6.2 mCi showed a partial remission for 1 year and a complete remission after a second low radiation dose. No acute toxicities were noted, and only myelotoxicity accompanied therapeutic doses, with grade IV marrow toxicity seen in three of seven patients receiving total doses of about 50 mCi. Dosimetry calculations showed spleen and tumor dose rules of about 4.6 cGy/mCi, which was three to four times the dose to other organs. Despite the administration of relatively low doses of LL2 (0.2 to 3.9 mg), 82% of 60 known extrasplenic lymphoma sites were imaged. Serum clearance showed an average distribution half-life (T1/2) of 2.1 hours and an elimination T1/2 of 32.0 hours. The average total-body clearance T1/2 was 43 to 45 hours. LL2's antigenic target does not appear to be shed in high amounts into the circulation. Three of eight patients having at least two injections showed a human antimouse antibody response. These patients may have been presensitized to animal protein. An interesting observation in this study was the marked drop in circulating B lymphocytes after the administration of radioiodinated LL2 or anticarcinoembryonic antigen MAbs, suggesting that this is a nonspecific radiation effect and not necessarily related to the binding of MAb to normal B cells.

Stein *et al.*, "Epitope specificity of the anti-(B cell lymphoma) monoclonal antibody, LL2," *Cancer Immunol Immunother.* 1993 Oct;37(5):293-8

LL2 is a murine monoclonal antibody IgG2a reactive with B cells and non-Hodgkin's B-cell lymphoma, which, in a radioiodinated form, induces responses in lymphoma patients [Goldenberg et al. (1991) *J Clin Oncol* 9:548-564]. In this report we identify LL2 as a member of the CD22 cluster. The molecular size of the antigen, its expression profile, and competitive blocking studies were used to establish this identification. By Western blot analysis and immunoprecipitation studies using the Raji Burkitt's lymphoma cell line metabolically labelled with [3H]leucine, the LL2 antigen was determined to correspond to a molecular mass of 140 kDa. The molecular mass of the LL2 antigen, and the B-cell-restricted reactivity of the LL2 antibody, were consistent with both the CD21 and CD22 clusters. To assess additional similarities and differences between LL2 and anti-CD22 and anti-CD21, the binding of these mAb to cultured cell lines, Nalm-6 and Molt-4, was compared by flow cytometry. The binding profile of LL2 on these cell lines was consistent with anti-CD22, but not anti-CD21. Sequential immunoprecipitation and cross-blocking studies with anti-CD22 monoclonal antibodies recognizing established CD22 epitopes were performed to confirm that LL2 reacts with CD22 and to determine which epitope LL2 recognizes. Binding of 131I-LL2 to Raji cells is inhibited over 90% by prior incubation of the target cells with unlabelled RFB4, indicating that LL2 belongs to the same epitope group as RFB4, *i.e.*, epitope B.

Epratuzumab: Targeting B-Cell Malignancies through CD22[1]

Morton Coleman,[2] David M. Goldenberg, Abby B. Siegel, Jamie C. Ketas, Michelle Ashe, Jennifer M. Fiore, and John P. Leonard

Center for Lymphoma and Myeloma, Division of Hematology/Oncology, Weill Medical College of Cornell University and New York Presbyterian Hospital, New York, New York 10021 [M. C.]; Garden State Cancer Center, Belleville, New Jersey 07109; and Immunomedics, Inc., Morris Plains, New Jersey 07950

Abstract

The development of effective B cell-directed monoclonal antibody therapies has dramatically altered the management of patients with B-cell non-Hodgkin's lymphoma. Anti-CD20 murine and chimeric antibodies have been characterized by manageable toxicity profiles and appear to have mechanisms which may be distinct from and complementary to those of chemotherapy. There is considerable rationale for treatment strategies which target other B-cell antigens, including CD22. This molecule is commonly expressed in non-Hodgkin's lymphoma and may mediate important functions in B-cell biology. Laboratory and initial clinical studies suggest that epratuzumab, a humanized anti-CD22 monoclonal antibody, may have antilymphoma activity in both unlabeled and radiolabeled forms. Efforts are underway to establish the utility of epratuzumab as a treatment for B-cell malignancies, through single agent and combination regimens, to define the optimal settings for its clinical application.

Introduction

NHL[3] is comprised of a heterogeneous group of malignancies which all represent neoplasms of lymphocytes. Although numerous NHL subtypes have been recognized, the most commonly observed entities are FL and LCL.

Chemotherapy, along with radiotherapy, has served as the mainstay of treatment for decades. The first significant departure from exclusive reliance on these therapeutics came from the FDA approval and introduction of rituximab, an unlabeled ("naked" or "cold") chimeric monoclonal antibody directed toward the CD20 antigen, which is anchored to the surface of most B-cell lymphoma cells. The demonstration that rituximab could produce ≥60% objective responses in low-grade NHL patients (depending on histology and previous therapy) with minimal toxicity has significantly impacted clinical practice and sparked enormous enthusiasm for this new modality of therapy (1–7). Subsequent studies have suggested that rituximab may be easily combined either sequentially or concurrently with chemotherapy, with acceptable side effects and potentially improved efficacy (8–11).

Although rituximab has proven especially beneficial to FL patients, a substantial proportion of patients do not respond, and almost every responding patient ultimately relapses, usually at a median of 9–12 months. Nevertheless, rituximab, representing proof of principle for the clinical application of antibodies in NHL, has prompted the search for new antibodies directed to other B cell lineage-specific antigen targets which may exhibit different patterns of tumor expression or may be associated with biological properties distinct from those of CD20.

CD22 as a Target for Immunotherapy

A variety of lymphocyte antigens is currently under evaluation as targets for immunotherapy. These include CD22, CD52, HLA-DR, CD80, and CD30. All have preliminarily exhibited encouraging results in preclinical or clinical studies (12–28). It has been hypothesized that antibodies against other lymphoma antigens may have antilymphoma effects that could overcome rituximab resistance or augment the activity of rituximab.

The CD22 antigen is a M_r 135,000 B-lymphocyte-restricted transmembrane sialoglycoprotein of the immunoglobulin superfamily. The predominant CD22 isoform contains seven extracellular domains (29, 30). CD22 is initially present in the cytoplasm of developing B cells but is later expressed on the surface during B-cell maturation once IgD expression occurs (31). Most circulating IgM+IgD+ cells express CD22. CD22 is strongly expressed in follicular (primary and secondary, B-cell zones), mantle, and marginal zone B cells but is weakly present in germinal (activated or differentiating) B cells. In B-cell malignancies, CD22 has been observed in ≥60–80% of samples evaluated (32). However, limited data are available with regard to expression of different CD22 isoforms in various NHL subtypes. When bound by ligand or antibody, CD22 is rapidly internalized within hours; internalization is "terminal," and reexpression is slow (days) (33, 34). The function of CD22 has not been entirely clarified, but reports have implicated a number of biological activities, including cellular adhesion and homing, as well as regulation of the B-cell activation (31, 35–38). Notably, CD22-deficient mice have mature B cells which may be more susceptible to apoptotic signals, have a shorter cellular life span, and are reduced in number in the bone marrow (39).

Potential Advantages of a Humanized Antibody Structure

Most monoclonal antibodies had been initially generated in a murine form and sometime later were modified to either chimeric (part murine and part human) or humanized forms.

[1] Presented at the "Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates," October 24–26, 2002, Princeton, NJ. Supported in part by a K23 award (to J. P. L) from the NIH (RR16814-02) and a pilot grant from the Cornell Center for Aging Research and Clinical Care (to J. P. L.).

[2] To whom requests for reprints should be addressed, at Weill Medical College of Cornell University, 407 East 70th Street, New York, NY 10021. E-mail: mortoncolemanmd@aol.com.

[3] The abbreviations used are: NHL, non-Hodgkin's lymphoma; FL, follicular lymphoma; LCL, large B-cell lymphoma.

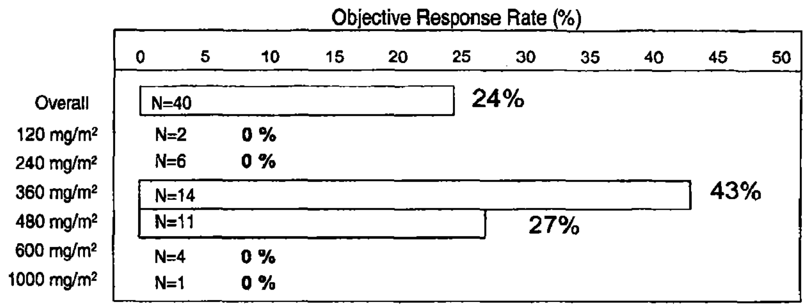


Fig. 1 Objective response rates by dose for evaluable patients with follicular NHL in Phase I/II study of epratuzumab in indolent NHL. The 95% confidence intervals were 11–40%, 18–71%, and 6–61% for the rates for overall, the 360 mg/m^2, and the 480 mg/m^2 groups, respectively. Reproduced from Leonard *et al.* (54) with permission from the authors and publisher.

Favorable characteristics of humanized antibodies may include a more extended half-life, which potentially allows for extended dosing intervals, and reduced immunogenicity, which confers value for multiple dose strategies. These qualities have potentially important relevance to dosing regimens and pharmacokinetics that may affect the therapeutic response and toxicity. Antibody structure may also impact the capability of an individual antibody to mediate effector cell killing through binding to Fc receptors (40). Various investigators are actively developing approaches which optimize antibody structure to improve engagement of the immune system to potentially improve antitumor effects. Other approaches include the addition of radioisotopes or toxins to antibodies to deliver additional modalities, which may also induce cell death (17, 19, 41–47).

Epratuzumab (Humanized LL2)

Epratuzumab is a humanized IgG1 monoclonal antibody directed against the CD22 antigen. Its parent murine antibody (LL2) has a broad range of reactivity against various B-cell lymphoma subtypes as demonstrated by immunohistochemistry (48), with little binding to normal tissue except for spleen. The antibody is rapidly internalized after attachment to CD22 (34). The murine LL2 antibody was subsequently re-engineered into the humanized (hLL2) epratuzumab (16). A number of different studies has been or are being conducted with both murine and humanized radiolabeled (Iodine-131 and Yttrium-90) forms of LL2, as well as with the unlabeled humanized form (epratuzumab; Immunomedics, Morris Plains, NJ, and Amgen, Thousand Oaks, CA). Laboratory investigations of putative mechanisms of action of epratuzumab as antilymphoma therapy are also underway. Antibody-dependent cellular cytotoxicity, as well as other pathways, may potentially be involved, similarly to those implicated in the activity of other therapeutic antibodies (49–52).

Clinical Studies of Unlabeled Epratuzumab (hLL2)

Although initial studies of epratuzumab focused on evaluation of radiolabeled constructs, several characteristics of unlabeled antibodies justify a parallel development strategy. Unlike radiolabeled agents, anti-B cell unlabeled antibodies are generally not associated with myelosuppression, facilitating treatment of patients with pre-existing cytopenias and extensive bone marrow involvement with tumor. Additionally, unlabeled antibodies are easier to combine with chemotherapy and other agents because of nonoverlapping toxicities. These attributes, in addition to the unique properties of the target CD22 antigen, all provided an impetus for the study of unlabeled epratuzumab in B-cell malignancies.

At the Center for Lymphoma and Myeloma at the Weill Medical College of Cornell University and the New York Presbyterian Hospital, we have explored the use of epratuzumab in patients with a variety of relapsed and refractory B-cell malignancies (53). An initial dose escalation trial used epratuzumab at i.v. doses from 120 to $\leq$1000 mg/m^2/week for four treatments, given generally over 30–60 min. Premedication with acetaminophen and diphenhydramine was provided to minimize the potential for infusion reactions. Treated subjects were heavily pretreated, with half having received four or more previous treatment regimens. Other adverse prognostic features were commonly present, including increased lactate dehydrogenase and tumor masses of $\geq$5 cm. Epratuzumab was very well tolerated across all dose levels examined, even when infused over $\leq$1 h. Infusion toxicities have been primarily grade 1 and manageable with usual supportive measures. Some patients exhibited transient B-cell depletion, but no other consistent laboratory abnormalities have been observed. No dose-limiting toxicity was observed, although for logistical reasons, escalation beyond 1000 mg/m^2/week was not performed. Among initial groups of FL, patients treated across all dose levels (n = 40), three complete and six partial responses, were preliminarily observed. Among the first group of LCL patients receiving epratuzumab, five objective responses were noted, including three complete responses. Overall responses appear to be more frequent around the 360 and 480 mg/m^2 dose levels (Fig. 1). Some of the responses have extended as long as several years. The tolerability and clinical activity in FL and LCL have suggested that further evaluation of this agent in NHL is warranted.

This preliminary evidence of antilymphoma activity led our group to study the concomitant use of epratuzumab and rituximab, to our knowledge the first study of combination antibody therapy in lymphoma. Because the mechanisms of action of the two agents may be different, and the targets are distinct, it is possible that the addition of epratuzumab may augment the activity of an anti-CD20-based regimen. Of course, one could theoretically postulate reduced efficacy of a combination, through a deleterious effect, although with disparate targets, a competition effect would be less likely. Additionally, the toxicity profile of a combination immunotherapy strategy

may be potentially more favorable relative than that of chemotherapy or radioimmunotherapy. Preliminary evaluation of the combination of epratuzumab and rituximab in B-cell NHL has yielded encouraging findings (54). Patients have received 360 mg/m^2 epratuzumab followed by 375 mg/m^2 rituximab weekly for four doses. Subjects have predominantly fallen into the FL and LCL categories, and early enrolling patients were rituximab naïve. Combination therapy was well tolerated with infusion-related toxicities National Cancer Institute grade I or II and comparable with those seen with antibody monotherapy. Objective responses have been demonstrated in the majority of patients (preliminarily 66% of follicular patients), and the quality of responses as reflected by complete responses and complete responses unconfirmed (preliminarily 60% in follicular patients) appear to be greater than expected with rituximab alone. Extended follow-up and additional accrual are necessary to validate our initial impressions, but these preliminary results suggest that this combination antibody regimen may be well tolerated and offers encouraging antilymphoma activity.

A number of other clinical trials with epratuzumab are either ongoing or expected, including studies of single agent epratuzumab in various B-cell lymphomas, multicenter evaluation of the epratuzumab and rituximab combination in FL and LCL, and studies of epratuzumab with chemotherapy, such as cyclophosphamide, doxorubicin, vincristine, and prednisone, with rituximab as primary therapy for LCL.

Conclusion and Future Directions

Considerable challenges still remain to elucidate the biology and function of epratuzumab and in the determination of the optimal setting for its use in B-cell malignancies. The evolving clinical data suggest that, ultimately, epratuzumab may have a significant role among the array of therapies available for lymphoma patients and will hopefully contribute to an improved therapeutic outlook for them.

Initial findings appear to indicate that epratuzumab has clinical activity as well as acceptable toxicity in FL and LCL. The unique characteristics of the CD22 target, and the properties of the humanized antibody structure, may contribute to these encouraging effects. Further evaluation of the *in vitro* and clinical characteristics of this treatment approach are under way, including more extended follow-up and additional patients among a variety of clinical studies. Although single agent activity of epratuzumab may be useful, a combination antibody therapy approach is particularly appealing, especially if toxicity is no greater, if the number and/or quality of responses ultimately prove to be better. While over the last several decades investigators have focused on combination chemotherapy strategies for lymphoma, we now have the option to potentially avoid the use of chemotherapy (and its associated toxicities) in certain clinical situations through use of combinations of biological agents, which specifically target malignant cells.

References

1. Maloney, D. G., Liles, T. M., Czerwinski, D. K., *et al.* Phase I clinical trial using escalating single dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood, *84:* 2457–2466, 1994.

2. Maloney, D. G., Grillo-Lopez, A. J., Bodkin, D. J., *et al.* IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J. Clin. Oncol., *15:* 3266–3274, 1997.

3. Maloney, D. G., Grillo-Lopez, A. J., White, C. A., *et al.* IDEC-C2B8: (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade lymphoma. Blood, *90:* 2188–2195, 1997.

4. McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J. Clin. Oncol., *16:* 2825–2833, 1998.

5. Piro, L. D., White, C. A., Grillo-Lopez, A. J., *et al.* Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. Ann. Oncol., *10:* 655–661, 1999.

6. Coiffier, B., Haioun, C., Ketterer, N., *et al.* Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood, *92:* 1927–1932, 1998.

7. Foran, J. F., Rohatiner, A. Z. S., Cunningham, D., *et al.* European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly-diagnosed mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. J. Clin. Oncol., *18:* 317–324, 2000.

8. Cruczman, M., Grillo-Lopez, A., White, C. A., *et al.* Progression-free survival (PFS) after six years (median) follow-up of the first clinical trial of rituximab/CHOP chemoimmunotherapy. Blood, *11:* 2518a, 2001.

9. Coiffier, B., Lepage, E., Briere, J., *et al.* CHOP chemotherapy plus Rituximab compared with CHOP alone in elderly patients with diffuse large B cell lymphoma. N. Engl. J. Med., *346:* 235–242, 2002.

10. Byrd, J. C., Peterson, B. L., Morrison, V. A., *et al.* Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712. Blood, *101:* 6–14, 2003.

11. Howard, O. M., Gribben, J., Neuberg, D. S., *et al.* Rituximab and CHOP induction therapy for newly-diagnosed mantle cell lymphoma: molecular complete responses are not predictive of progression-free survival. J. Clin. Oncol., *20:* 1288–1294, 2002.

12. Dyer, M. J. The role of CAMPATH-1H antibodies in the treatment of lymphoid malignancies. Semin. Oncol., *5* (Suppl. 14): 52–57, 1999.

13. Khorana, A., Bunn, P., McLaughlin, P., *et al.* A phase II multicenter study of CAMPATH-1H antibody in previously-treated patients with non bulky non-Hodgkin's lymphoma. Leuk. Lymphoma, *41:* 77–87, 2001.

14. Keating, M. J., Flynn, I., Jain, V., *et al.* Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood, *99:* 3554–3561, 2002.

15. Rai, K. R., Freter, C. E., Mercier, R. J., *et al.* Alemtuzumab in previously treated chronic lymphocytic leukemia patients who had also received fludarabine. J. Clin. Oncol., *20:* 3891–3897, 2002.

16. Leung, S. O., Goldenberg, D. M., Dion, A. S., *et al.* Construction and characterization of a humanized, internalizing B cell (CD22)-specific, leukemia/lymphoma antibody, LL2. Mol. Immunol., *32:* 1413–1427, 1995.

17. Goldenberg, D. M., Horowitz, J. A., Sharkey, R. M., *et al.* Targeting, dosimetry, and radioimmunotherapy of B-cell lymphoma with iodine-131-labelled LL2 monoclonal antibody. J. Clin. Oncol., *9:* 548–564, 1991.

18. Juweid, M., Sharkey, R. M., Markowitz, A., *et al.* Treatment of non-Hodgkin's lymphoma with radiolabelled murine, chimeric or humanized LL2, an anti-CD-22 monoclonal antibody. Cancer Res., *55:* 5899s–5907s, 1995.

19. Vose, J. M., Colcher, D., Gobar, L., *et al.* Phase I/II trial of multiple dose 131 iodine-Mab LL2 (CD22) in patients with recurrent non-Hodgkin's lymphoma. Leuk. Lymphoma, *38:* 91–101, 2000.

20. Lin, T. S., Stock, W., Lucas, M. S., *et al.* A Phase I dose-escalation study of apolizumab (Hu1D10) using a stepped-up dosing schedule in

patients with Chronic Lymphocytic Leukemia (CLL) and Acute Lymphocytic Leukemia (ALL). Blood, 802a, 2002.

21. Link, B. K., Wang, H., Byrd, J.C., *et al.* Phase I study of Hu1D10 monoclonal antibody (Remitogen ™) in patients with B-cell lymphoma. Proc. Am. Soc. Clin. Oncol., 284a, 2001.

22. Brown, K. S., Levitt, D. J., Shannon, M., *et al.* Phase II trial of Remitogen ™(Humanized ID10) Monoclonal antibody targeting class II in patients with relapsed low-grade or follicular lymphoma. Clinical Lymphoma, *2:* 188–190, 2002.

23. Nagy, Z. A., Hubner, B., Lohning, C., *et al.* Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. Nat. Med., *8:* 801–807, 2002.

24. Bolognesi, A., Polito, L., Tazzari, P. L. *et al.* In vitro anti-tumor activity of anti-CD80 and anti-CD86 immunotoxins containing type I ribosome-inactivating proteins. Br. J. Hematol., *110:* 351–361, 2000.

25. Hariharan, K., Anderson, D., Leigh, B., *et al.* Therapeutic activity of IDEC-114 (Anti CD80) and rituximab (Rituxan®) in B cell lymphoma. Blood, 608a, 2001.

26. Czuczman, M. S., Witzig, T. E., Younes, A., *et al.* IDEC-114, an anti-CD80 monoclonal antibody for relapsed or refractory, follicular NHL: a phase I/II study of safety, efficacy and pharmacokinestics. Blood, 163a, 2002.

27. Bartlett, N., Younes, A., Carabasi, M. A., *et al.* Phase I study of SGN-30, a chimeric monoclonal antibody (mAb), in patients with refractory or recurrent CD30+ hematologic malignancies. Blood, 362a, 2002.

28. Borchmann, P., Schnell, R., Fuss, I., *et al.* Phase I trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood, *100:* 3101–3107, 2002.

29. Engel, P., Nojima, Y., Rothstein, D., *et al.* The same epitope on CD22 of B lymphocytes mediates the adhesion of erythrocytes, T and B lymphocytes, neutrophils, and monocytes. J. Immunol., *15:* 4719–4732, 1993.

30. Powell, L. D., and Varki, A. I-type lectins. J. Biol. Chem., *270:* 14243–14246, 1995.

31. Tedder, T. F., Tuscano, J., Sato, S., *et al.* CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. Annu. Rev. Immunol., *15:* 481–504, 1997.

32. Cesano, A., Gayko, U., Brannan, C., Kapushoc, H., Fields, S. Z., and Perkins, S. L. Differential expression of CD22 in indolent and aggressive non-Hodgkin's lymphoma (NHL): implications for target immunotherapy. Blood, 350a, 2002.

33. Shan, D., and Press, O. W. Constitutive endocytosis and degradation of CD22 by human B cells. J. Immunol., *154:* 4466–4475, 1995.

34. Shih, L. B., Lu, H. H., Xuan, H., and Goldenberg D. M. Internalization and intracellular processing of an anti-B cell lymphoma monoclonal antibody, LL2. Int. J. Cancer, *56:* 538–545, 1994.

35. Peaker, C. J., and Neuberger, M. S. Association of CD22 with the B cell antigen receptor. Eur. J. Immunol., *23:* 1358–1363, 1993.

36. Nath, D., van Der Merwe, P. A., Kelm, S., *et al.* The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22. J. Biol. Chem., *270:* 26184–26191, 1995.

37. Sgroi, D., Koretsky, G. A., and Samenkovic, I. Regulation of CD45 engagement by the B cell receptor CD22. Proc. Natl. Acad. Sci. USA, *92:* 4026–4030, 1995.

38. Kelm, S., Pelz, A., Schauer, R., *et al.* Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. Curr. Biol., *4:* 965–972, 1994.

39. Nitschke, L., Carsetti, R., Ocker, B., *et al.* CD22 is a negative regulator of B-cell receptor signaling. Curr. Biol., *7:* 133–143, 1997.

40. Clynes, R. A., Towers, T. L., Presta, L. G., *et al.* Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor target. Nat. Med., *6:* 443–446, 2000.

41. Juweid, M., Sharkey, R. M., Markowitz, A., *et al.* Treatment of non-Hodgkin's lymphoma with radiolabelled murine, chimeric or humanized LL2, an anti-CD-22 monoclonal antibody. Cancer Res., *55:* 5899s–5907s, 1995.

42. Linden, O., Tennvall, J., Cavallin-Stahl, E., *et al.* Radioimmunotherapy using 131I-labelled anti-CD22 monoclonal antibody (LL2) in patients with previously-treated B-cell lymphomas. Clin. Cancer Res., *5:* 3287s–3291s, 1999.

43. Juweid, M. E., Stadtmauer, E., Haggar, G., *et al.* Pharmacokinetics, dosimetry and initial therapeutic results with (131I) and (111)IN/90Y-labeled humanized LL2 antI-CD22 monoclonal antibody in patients with relapsed, refractory NHL. Clin. Cancer Res., 5:3292s–3303s, 1999.

44. Hajjar, G., Burton, J., Sharkey, R. M., *et al.* Phase I/II radioimmunotherapy trial with (90Y)-labelled epratuzumab (LymphoCide; anti-CD22 monoclonal antibody) in relapsed/refractory non-Hodgkin's lymphoma (NHL). J. Nucl. Med., *42s:* 156P, 2001.

45. Linden, O., Tennvall, J., Cavallin-Stahl, E., *et al.* Durable response to 90-yttrium-epratuzumab (hLL2) in B-cell lymphoma failing chemotherapy by using dose-fractionation schedule. Blood, 602a, 2001.

46. Behr, T. M., Wormann, B., Gramatzki, M., *et al.* Low-versus high-dose radioimmunotherapy with humanized anti-CD22 or chimeric anti CD20 antibodies in a broad spectrum of B cell-associated malignancies. Clin. Cancer Res., *5:* 3304s–3314s 1999.

47. Postema, E. J., Mandigers, C. M. P. W., Corstens, F. H. M., *et al.* Final results of a phase I immunotherapy trial using ^{186}Re-epratuzumab for the treatment of patients with non-Hodgkin's lymphoma. Cancer Biother. Radiopharm., *17:* 491, 2002.

48. Pawlak-Byczkowska, E. J., Hansen, H. J., Dion, A. S., and Goldenberg, D. M. Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma. Cancer Res., *49:* 4568–4577, 1989.

49. Byrd, J. C., Kitada, S., Flinn, I. W., *et al.* The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood, *99:* 1038–1043, 2002.

50. Golay, J., Zaffaroni, L., Vaccari, T., *et al.* Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituxumab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood, *95:* 3900–3908, 2000.

51. Shan, D., Ledbetter, J. A., and Press, O. W. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood, *91:* 1644–1652, 1998.

52. Leonard, J. P., Coleman, M., Matthews, J. C., *et al.* Phase I/II trial of Epratuzumab (humanized anti CD-22 Antibody) in non-Hodgkin's lymphoma. Blood, 358a, 2002.

53. Leonard, J. P., Coleman, M., Ketas, J. C., *et al.* Phase I/II trial of Epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. J. Clin. Oncol., in press, 2003.

54. Leonard, J. P., Coleman, M., Matthews, J. C., *et al.* Epratuzumab (Anti-CD22) and rituximab (anti-CD20) combination immunotherapy for non-Hodgkin's lymphoma: preliminary response data. Proc. Am. Soc. Clin. Oncol., 266a, 2002.

Available online http://arthritis-research.com/content/8/3/R74

Research article **Open Access**

Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus

Thomas Dörner[1], Joerg Kaufmann[1], William A Wegener[2], Nick Teoh[2], David M Goldenberg[2,3] and Gerd R Burmester[1]

[1]Department of Medicine/Rheumatology and Clinical Immunology, Charite Hospital, Berlin, Germany
[2]Immunomedics, Inc., Morris Plains, NJ, USA
[3]Center for Molecular Medicine and Immunology, Belleville, NJ, USA

Corresponding author: Thomas Dörner, thomas.doerner@charite.de

Received: 2 Nov 2005 Revisions requested: 4 Jan 2006 Revisions received: 21 Mar 2006 Accepted: 22 Mar 2006 Published: 21 Apr 2006

Arthritis Research & Therapy 2006, **8**:R74 (doi:10.1186/ar1942)
This article is online at: http://arthritis-research.com/content/8/3/R74

Abstract

B cells play an important role in the pathogenesis of systemic lupus erythematosus (SLE), so the safety and activity of anti-B cell immunotherapy with the humanized anti-CD22 antibody epratuzumab was evaluated in SLE patients. An open-label, single-center study of 14 patients with moderately active SLE (total British Isles Lupus Assessment Group (BILAG) score 6 to 12) was conducted. Patients received 360 mg/m^2 epratuzumab intravenously every 2 weeks for 4 doses with analgesic/antihistamine premedication (but no steroids) prior to each dose. Evaluations at 6, 10, 18 and 32 weeks (6 months post-treatment) follow-up included safety, SLE activity (BILAG score), blood levels of epratuzumab, B and T cells, immunoglobulins, and human anti-epratuzumab antibody (HAHA) titers. Total BILAG scores decreased by $\geq$ 50% in all 14 patients at some point during the study (including 77% with a $\geq$ 50% decrease at 6 weeks), with 92% having decreases of various amounts continuing to at least 18 weeks (where 38% showed a $\geq$ 50% decrease). Almost all patients (93%) experienced improvements in at least one BILAG B- or C-level disease activity at 6, 10 and 18 weeks. Additionally, 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks. Epratuzumab was well tolerated, with a median infusion time of 32 minutes. Drug serum levels were measurable for at least 4 weeks post-treatment and detectable in most samples at 18 weeks. B cell levels decreased by an average of 35% at 18 weeks and remained depressed at 6 months post-treatment. Changes in routine safety laboratory tests were infrequent and without any consistent pattern, and there was no evidence of immunogenicity or significant changes in T cells, immunoglobulins, or autoantibody levels. In patients with mild to moderate active lupus, 360 mg/m^2 epratuzumab was well tolerated, with evidence of clinical improvement after the first infusion and durable clinical benefit across most body systems. As such, multicenter controlled studies are being conducted in broader patient populations.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that can involve many organ systems [1]. In Europe and the United States, estimates of the number of affected individuals range from 24 to 65 cases per 100,000 people [1,2]. The clinical course of SLE is episodic, with recurring activity flares causing increasing disability and organ damage. Cyclophosphamide, azathoprine, and corticosteroids remain important for long-term management of most patients having active disease, and even those in clinical remission [1]. Despite the important advances made with these drugs, especially cyclophosphamide, in controlling lupus disease activity, they have considerable cytotoxicity and cause, for example, bone marrow depression, ovarian failure, enhanced risk of bladder cancer, as well as the known side effects of long-term systemic corticosteroid therapy. As such, there continues to be a need for the development of targeted and less toxic therapies.

BCR = B cell antigen receptor; BILAG = British Isles Lupus Assessment Group; HACA = human anti-chimeric antibody; HAHA = human anti-human (epratuzumab) antibody; NCI CTC = National Cancer Institute Common Toxicity Criteria; NHL = non-Hodgkin lymphoma; SLE = systemic lupus erythematosus.

Specific autoantibodies against nuclear, cytoplasmic, and membrane antigens remain the serological hallmark of SLE. While lymphopenia is common, there is an increase in the level of activated B cells [3,4] and characteristic alterations of B cell subpopulations [5,6] that may be driven by extrinsic or intrinsic factors. B cells appear to have a key role in the activation of the immune system, in particular through the production of cytokines and by serving as antigen-presenting cells (reviewed recently in [7]). Although B cell activation can occur independently of T cell help in lupus, a substantial fraction of B cells is activated in a T cell dependent manner [8-10], as demonstrated by isotype switching and affinity maturation of B cells [11,12] and enhanced CD154-CD40 interactions [13]. Useful insight into the pathogenesis of lupus has been obtained with animal models. MRL/lpr mice spontaneously develop a lupus-like autoimmune disease in an age-dependent manner, including autoantibody production, arthritis, skin lesions, and severe nephritis, which usually leads to early demise from renal failure [14]. When rendered B cell deficient, they no longer develop nephritis, mononuclear infiltrates are no longer detectable in the kidneys or skin, the number of activated memory T cells are markedly reduced, and infusions of pooled serum from diseased MRL/lpr mice lead to glomerular antibody deposition, but not the development of renal disease [15,16]. However, when reconstituted with B cells not able to secrete circulating antibodies, they develop nephritis and vasculitis [17]. As such, it appears that B cells play a direct role in promoting disease beyond the production of autoantibodies [18].

Depleting B cells with anti-CD20 monoclonal antibodies has emerged as a potentially new therapeutic strategy for certain autoimmune diseases. The chimeric monoclonal antibody rituximab depletes B cells by targeting the pan-B cell surface antigen CD20. Preliminary experience with rituximab in about 100 patients with SLE (recently reviewed in [7]) and other autoimmune diseases has been encouraging [6,19-22].

Due to the central role of B cells in the pathogenesis of certain autoimmune diseases, targeted anti-B cell immunotherapies would be expected to offer therapeutic value in the setting of SLE. In addition to CD20, another unique target is CD22, a 135 kDa glycoprotein that is a B-lymphocyte-restricted member of the immunoglobulin superfamily, and a member of the sialoadhesin family of adhesion molecules that regulate B cell activation and interaction with T cells [23-27]. CD22 has seven extracellular domains and is rapidly internalized when cross-linked with its natural ligand, producing a potent costimulatory signal in primary B cells [25,28-30]. The function of CD22 in cell signaling is suggested by six tyrosine and three inhibitory domain sequences in the intra-cellular cytoplasmic tail. These inhibitory domains are phosphorylated by the non-receptor kinase Lyn upon B cell antigen receptor (BCR) activation by IgM ligation, leading to the activation and recruitment of SHP-1 phosphatase [31,32]. SHP-1 is a tyrosine phosphatase that negatively regulates several intracellular signaling pathways, including the calcium pathway, through dephosphorylation of signaling intermediates, such as Lyn and Syk. CD22 is first expressed in the cytoplasm of pro-B and pre-B cells, and then on the surface of B cells as they mature, with expression ceasing with B cell differentiation into plasma cells [23]. Studies in CD22-deficient mice and in CD22-negative cell lines have shown an increase in calcium response to BCR ligation [33-36], indicating that CD22 inhibition of BCR signaling is achieved through the mechanism of controlling calcium efflux in B cells. It has been reported that this effect of CD22 is mediated by potentiation of plasma membrane calcium-ATPase and requires SHP-1 [37]. Animal experiments indicate that CD22 plays a key role in B cell development and survival, with CD22-deficient mice having reduced numbers of mature B cells in the bone marrow and circulation, and with the B cells also having a shorter life span and enhanced apoptosis [31].

Therefore, CD22 is an attractive molecular target for therapy because of its restricted expression; it is not exposed on embryonic stem or pre-B cells, nor is it normally shed from the surface of antigen-bearing cells. Initially, a mouse monoclonal antibody (mLL2, formerly EPB-2) was developed and characterized that specifically binds to the third domain of CD22 [38,39]. Immunohistological evaluation revealed that it recognized B cells within the spleen and lymph nodes, but did not react with antigen unrelated to B cells in normal and solid tumor tissue specimens, and flow cytometry showed no reactivity with platelets, red blood cells, monocytes, and granulocytes in normal peripheral blood [38,39]. The complementarity-determining regions of mLL2 were subsequently grafted onto a human IgG_1 genetic backbone [40]. Epratuzumab, the resulting complementarity-determining region-grafted (recombinant) 'humanized' monoclonal antibody (hLL2), is 90% to 95% of human origin, thus greatly reducing the potential for immunogenicity. Epratuzumab has been shown to mediate antibody-dependent cellular cytotoxicity *in vitro*[41] , and may also exhibit biological activity through modulating BCR function (J Carnahan, R Stein, Z Qu, K Hess, A Cesano, HJ Hansen, DM Goldenberg, manuscript submitted).

In clinical trials, over 400 patients with non-Hodgkin lymphoma (NHL) or other B cell malignancies have received epratuzumab administered as 4 consecutive weekly infusions over about 60 minutes. An initial phase I/II study administered doses of up to 1,000 mg/m^2, with patients premedicated each week with oral acetaminophen and diphenhydramine to minimize potential infusion reactions. Epratuzumab toxicity consisted primarily of mild to moderate transient infusion-related events during the first infusion, and only one patient with a prior right lung resection for a fungal abscess had a serious event (bronchospasm during infusion), which was treated with parenteral medications. Based on this safety record, objective evidence of tumor

response, and less severe depression of circulating B cells [42,43], 4 consecutive weekly doses of 360 mg/m^2 epratuzumab was selected as a sufficiently safe and efficacious treatment regimen to warrant further clinical development. A pharmacokinetic analysis of weekly dosing subsequently demonstrated that the post-treatment serum half-life of epratuzumab in NHL patients was 19 to 25 days, consistent with the half-life of a human IgG_1 [44]. As such, a longer interval between doses was indicated, and a biweekly dosing schedule was selected for this initial study in SLE. We report here the first experience of treating an autoimmune disease with a CD22 antibody, epratuzumab.

Materials and methods

This initial, phase II, open-label, non-randomized, single-center study was undertaken to obtain preliminary evidence of therapeutic activity in SLE, to confirm the safety, tolerance and lack of immunogenicity of epratuzumab in this population, and to evaluate pharmacokinetic and pharmacodynamic parameters. The study was approved by the Ethics Committee of Charité University Hospital.

Patient population

Males or non-pregnant, non-lactating females, ≥ 18 years of age, were eligible to participate provided they had a diagnosis of SLE according to the American College of Rheumatology revised criteria (fulfilled ≥ 4 criteria), with SLE for at least 6 months, and at least one elevated autoantibody level (antinuclear antibodies/ANA and/or anti-dsDNA) and moderately active disease (a score of 6 to 12 for total British Isles Lupus Assessment Group (BILAG) disease activity) at study entry. Patients were excluded if they had prior rituximab or other antibody therapy, allergies to murine or human antibodies, experimental therapy within 3 months, active severe CNS (central nervous system) lupus, laboratory abnormalities (hemoglobin < 8.0 g/dl, WBC (white blood cells) < 2,000/mm^3, ANC (absolute neutrophil cells) < 1,500/mm^3, platelets < 50,000/μl, liver transaminases or alkaline phosphatase more than twice upper limit of normal, serum creatinine > 2.5 mg/dl, or proteinuria > 3.5 gm/day), thrombosis, drug or alcohol abuse, infection requiring hospitalization within 3 months, long-term active infectious diseases (tuberculosis, fungal infections) within 2 years, malignancy (except basal cell carcinoma, cervical carcinoma in situ (CIS), history of recurrent abortions (2 or more), or known HIV, hepatitis B or C, or other immunosuppressive states.

Concomitant medications

Pulsed methylprednisolone, other high-dose corticosteroids, cyclophosphamide, and intravenous, joint, or intramuscular corticosteroid injections were not allowed during the study or within four weeks of study entry. Low-dose corticosteroids (prednisone, = 20 mg/day or equivalent) or background therapy with standard antirheumatic immunosuppressives (for example, azathioprine, methotrexate) was permitted provided there were no dosing changes during the study or within four weeks prior to study entry. Antimalarials, non-steroidal anti-inflammatory drugs (NSAIDs), ACE-inhibitors or angiotensin receptor antagonists were also allowed, provided there were no dosing changes during the study or within two weeks of study entry.

Treatment schedule

After satisfying eligibility, signing informed consent, and undergoing baseline evaluations, all patients received 4 doses of 360 mg/m^2 epratuzumab administered every other week with paracetamol (acetaminophen) and an antihistamine (but no steroids) given as premedication prior to each dose.

Study evaluations

The BILAG system was used to categorize the severity level of lupus disease activity in each patient at study entry and at post-treatment evaluations obtained at 6 (24 hours after the last infusion), 10 and 18 weeks and at an additional 32 weeks (6 month post-treatment) follow-up visit. The BILAG system organizes lupus-associated signs and symptoms according to eight body systems: general/constitutional, mucocutaneous, neurological, musculoskeletal, cardiovascular/respiratory, vasculitic, renal, hematological domains [45,46]. At each evaluation, the presence and change of any signs and symptoms were recorded and the level of any disease activity within each body system determined on a treatment-intent basis, according to BILAG rules as: A (severely active disease sufficient to require disease-modifying treatment, for example, > 20 mg/d prednisolone, immunosuppressants/cytoxics); B (moderately active disease requiring only symptomatic therapy, for example < 20 mg/d prednisolone, antimalarials, NSAIDs alone or in combination); or C (stable mild disease with no indication for changes in treatment). To assign an overall disease activity level for each patient, a total BILAG score was determined by adding a numerical severity score (A = 9, B = 3, C = 1, no activity = 0) across the eight body systems. Other evaluations at these times included an SLE panel (autoantibodies, C3, C-reactive protein/CRP, erythrocyte sedimentation rate/ESR, other laboratory tests), vital signs, physical examination, adverse events, routine safety laboratory tests (hematology, serum chemistry), urinalysis, serum immunoglobulins, peripheral blood B and T cells, epratuzumab serum levels (analyzed by sponsor), and human anti-human (epratuzumab) antibody titers (HAHA; analyzed by sponsor).

Human anti-human (epratuzumab) antibody assay

The sponsor's HAHA test is a competitive ELISA assay, where the capture reagent is epratuzumab and the probe is an anti-epratuzumab-idiotype antibody. The anti-idiotype antibody is an acceptable surrogate for what is reacted against in an immunogenic response by humans against the binding portion of epratuzumab that distinguishes the molecule from other human antibodies (for instance, the framework region that has human amino acid sequences). Test results are derived from

Table 1

Number of patients with B-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ constitutional	3	Fatigue/malaise/lethargy (3)
		Anorexia/nausea/vomiting (2)
		Unintentional weight loss > 5% (1)
II. Mucocutaneous	13	Malar erythema (11)
		Active localized discoid lesions (2)
		Mild maculopapular eruption (1)
III. Neurological	0	
IV. Musculoskeletal	2	Arthritis (2)
V. CV/Respiratory	2	Dyspnea (2)
		Pleuropericardial pain (2)
VI. Vasculitis	5	Minor cutaneous vasculitis (nailfold/digital vasculitis, purpura, urticaria) (5)
VII. Renal	0	
VIII. Hematology	1	Anemia (hemoglobin < 11 g/dL) (1)

*Signs and symptoms that contributed to the B-level disease activity according to BILAG rules.

an eight-point standard curve with varying dilutions of anti-idiotype antibody in bovine serum albumin. Patient serum samples are diluted 1:2 with bovine serum albumin and assayed in triplicate. The anti-idiotype standard curve is used to determine the presence of HAHA in unknown samples. An acceptable assay is based on linear regression parameters that must be met to define a valid assay.

Statistical analyses

The primary assessment of disease activity compared post-treatment BILAG results with those at study entry, using total BILAG scores for overall assessment and letter grade categories to assess the level of disease activity within each body system. Adverse events and safety laboratory tests were graded according to NCI CTC version 3.0 criteria on a 1 to 4 scale for toxicity (1, mild; 2, moderate; 3, severe; 4, life threatening). All analyses of efficacy, safety, tolerance, immunogenicity, pharmacokinetics, and pharmacodynamics used descriptive statistics. Wilcoxon signed rank test was used to assess the statistical significance of changes in total BILAG scores compared to their baseline values. All statistical tests used a significance level of 0.05.

Table 2

Number of patients with C-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ Constitutional	11	Fatigue/malaise/lethargy (10)
		Anorexia/nausea/vomiting (1)
		Lymphadenopathy/splenomegaly (1)
		Pyrexia (documented) (1)
II. Mucocutaneous	1	Mild alopecia (1)
III. Neurological	10	Episodic migrainous headaches (8)
		Severe, unremitting headache (2)
IV Musculoskeletal	11	Arthralgia (10)
		Myalgia (9)
		Improving arthritis (1)
V. CV/ Respiratory	2	Dyspnea (1)
		Pleuropericardial pain (1)
VI. Vasculitis	4	Raynaud's (3)
		Livido reticularis (1)
VII. Renal	4	Mild/stable proteinuria (4)
VIII. Hematology	11	Lymphocytopenia (< 1500 cells/μl) (10)
		Evidence of circulating anticoagulant (1)
		Decreased platelets (< 150,000/μl) (1)

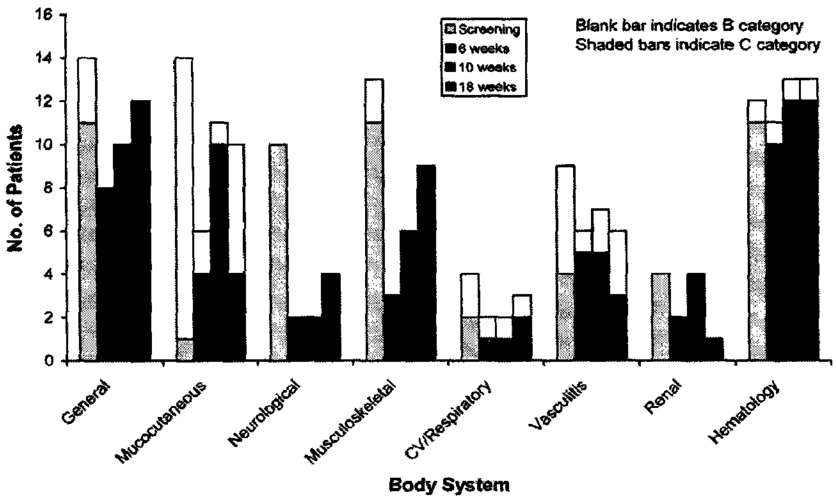
*Signs and symptoms that contributed to the C-level disease activity according to BILAG rules.

Results

Demographics and patient characteristics at study entry

A total of 14 Caucasian patients (13 females and 1 male; 23 to 53 years old, median age 40 years) were enrolled. At study entry, the patients had been initially diagnosed with SLE 1 to 19 years (median 10 years) earlier and were receiving corticosteroids ($n = 13$, 1 to 12 mg/day prednisolone) plus immunosuppressives ($n = 11$, including 50 to 200 mg/day azathioprine, $n = 9$; 20 mg/day methotrexate, $n = 2$; 2 g/day mycophenalate mofetil, $n = 1$), and antimalarials ($n = 6$, 200 to 600 mg/day hydroxychloroquine). All patients had positive ANA at study entry (titers of 80:1 to 5,120:1), and 5 patients (36%) had positive anti-dsDNA antibody levels (> 10 U/ml). Ten patients (71%) had ESR values that were elevated (> 15 mm/h) and 4 patients (29%) had raised CRP levels (> 0.5 mg/dl), while only 3 patients (21%) had C3 levels that were borderline low or decreased (< 90 mg/dl), and no patient had

Figure 1



Frequency comparison of BILAG B- and C-level activities for each body system at screening, 6, 10 and 18 weeks.

positive direct Coombs' or serum haptoglobulin levels elevated above borderline.

All patients had total BILAG scores of 6 to 12 (median 10) at study entry. No patient had A-level disease activity in any body system, 13 patients had B-level disease activity in at least one body system (2 with three Bs, 9 with 2 Bs, 2 with one B) and one patient had only C-level activities. B-level disease occurred primarily in the mucocutaneous, vasculitis, and general/constitutional body systems, with no B-level disease activity in the neurological or renal systems (Table 1), while C-level disease occurred primarily in the general/constitutional, musculoskeletal, hematological and neurological body systems (Table 2). The actual signs and symptoms at study entry that contributed to the B-level disease activity according to the BILAG rules are also summarized in Table 1, while those contributing to C-level disease activity are summarized in Table 2.

Study drug administration

Twelve of the 14 patients (86%) completed all 4 infusions of 360 mg/m^2 epratuzumab as scheduled, while one patient with sleepiness attributed to premedication IV antihistamines prematurely terminated the first infusion but subsequently completed all 3 remaining infusions without further event, and one patient completed the first two infusions, but discontinued further infusions after development of herpes zoster, which responded to antivirals. The infusions were well tolerated, with a median infusion time of 32 minutes (23 to 86 minutes), and with infusion reactions in 6 patients all limited to occurrences of transient, mild (grade 1 NCI toxicity) adverse events (flu-like symptoms, tracheitis/throat ache, $n = 2$; arthralgia/myalgia, fever, fatigue, nausea, headache, chills, or rash, $n = 1$).

Post-treatment evaluations and follow-up

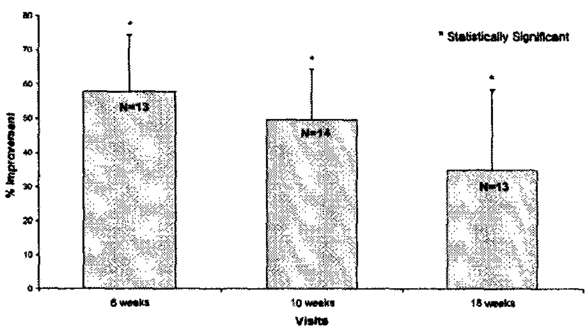
All patients remained in the study through the 18-week post-treatment evaluation period. One patient had a late 18-week visit that fell within the 32-week time frame and the corresponding data were hence re-assigned to the 32-week visit. The single patient who did not complete all 4 infusions continued to receive post-treatment evaluations beginning at the 10-weeks follow-up visit. Except for the aforementioned deviations, all patients received post-treatment evaluations at 6, 10, and 18 weeks. One patient was lost to follow-up after 18 weeks, while 13 patients returned for the final 32-week evaluations (8 patients as scheduled, 5 with a delayed visit between 42 to 82 weeks).

BILAG treatment response

The effect of epratuzumab on clinical manifestations was evaluated at 6, 10, and 18 weeks using numerical total BILAG scores as well as categorical scores. The compositions of B- and C-level activities improved after treatment, primarily in the general, mucocutaneous and musculoskeletal systems (Figure 1). Improvement in C-level activity was also observed in the neurological and renal domains. Improvements in the general, mucocutaneous, neurological and musculoskeletal systems occurred earlier compared to the cardiovascular/respiratory, vasculitic and renal systems (Figure 2). However, the limited number of patients with manifestations in each of these systems precludes a definitive determination of preferential effects. In terms of changes in the total BILAG score, statistically significant improvement was observed at 6, 10, and 18 weeks (Figure 3). Additionally, a substantial proportion of patients showed 50% or more improvement in total BILAG score at weeks 6, 10, and 18 (77%, 71% and 38%, respectively). At the final 32-week evaluation, statistically significant

Figure 2

Level of Improvement	6 weeks	10 weeks	18 weeks
Pts with Decreased Scores	100% (13/13)	100% (14/14)	92% (12/13)
Pts with Decreases ≥ 50%	77% (10/13)	71% (10/14)	38% (5/13)



Overall frequency and mean improvement of total disease activity as measured by the total BILAG score at 6, 10 and 18 weeks.

improvement in total BILAG score continued to be observed, with 15% of the patients achieving 50% or more improvement.

In a separate analysis, the total number of patients who achieved BILAG improvements in the particular domains at 6, 10 and 18 weeks of follow-up are summarized in Table 3. This indicates that the most characteristic BILAG domains, as also seen in Figure 2, were more likely to respond, although the duration of response was very similar throughout the domains. In fact, deterioration in BILAG categorical scores compared to baseline was infrequently seen during the study (Table 4). Only two patients (14%) showed worsening of hematological parameters (lymphocytopenia), one starting at 6 weeks and the other at 18 weeks. Another patient manifested renal (mild proteinuria) deterioration at 10 weeks. Overall, at week 18, 3 patients (21%) had a deteriorated BILAG assessment in at least one body system compared to baseline.

Table 3

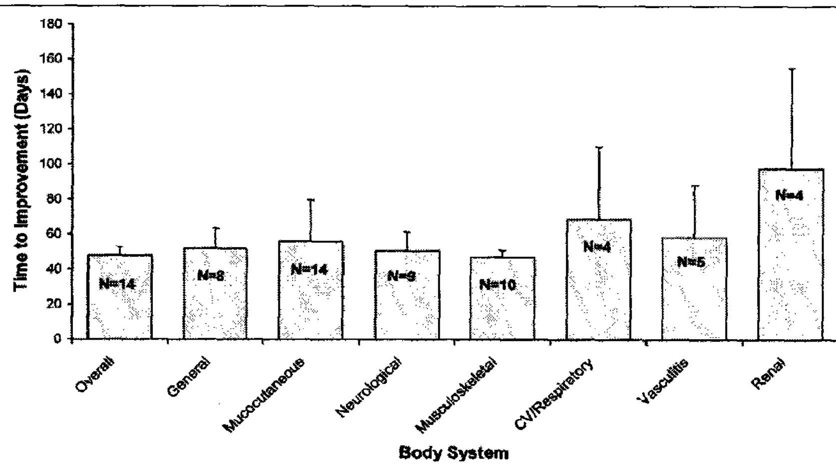
Number of patients with improvement from baseline BILAG B- and C-level activities

BILAG body system	6 weeks[a]	10 weeks	18 weeks
General (N = 14)[b]	6 (43%)	5 (36%)	2 (14%)
Mucocutaneous (N = 14)	11 (79%)	8 (57%)	6 (43%)
Neurological (N = 10)	7 (70%)	8 (80%)	6 (60%)
Musculoskeletal (N = 13)	9 (69%)	7 (54%)	4 (31%)
CV/Respiratory (N = 4)	3 (75%)	3 (75%)	3 (75%)
Vasculitis (N = 9)	4 (44%)	3 (33%)	3 (33%)
Renal (N = 4)	2 (50%)	1 (25%)	3 (75%)
Hematology (N = 12)	0 (0 %)	0 (0 %)	0 (0 %)
Overall[c] (N = 14)	13 (93%)	14 (100%)	13 (93%)

[a]Twenty-four hours after fourth infusion. [b]N = number of patients with involvement in a particular body system at entry. [c]As applied to any BILAG body system.

An additional analysis was performed to determine the durability of resolution of certain B- and C-level activities (Table 5). Although in a number of patients, B- and C-level activities resolved persistently, the heterogeneity of patients' manifesta-

Figure 3



Mean time to improvement of each BILAG body stystem. Mean time to improvement (in days) of each BILAG body system during the follow-up of the study (N denotes the number of patients available for analysis for each body system). Since the first evaluation was scheduled for 6 weeks, the earliest time to improvement is at least 42 days.

tions again precluded the identification of a preferential response profile to the drug.

Safety

During or following treatment, a total of ten patients reported adverse events. As reported above, six had mild, transient, infusional reactions and one patient experienced somnolence following antihistamine medication. Subsequently, five patients had infections (including herpes zoster, otitis media, *Helicobacter pylori*-associated gastritis, vaginitis/vaginal candidiasis, cystitis, and tonsillitis) that resolved with appropriate treatment, and one patient had spinal contusion from a traffic accident. Standard safety laboratory tests showed no consistent pattern of change from baseline, and infrequent post-treatment increases in NCI CTC v3.0 toxicity grades for these laboratory tests were all limited to changes of one grade level except for one patient with an increase in lymphocytes from grade 1 to grade 3, and another from grade 0 to grade 3 (Table 6).

Pharmacokinetics and immunogenicity

Of the 14 patients, serum samples for analysis of pharmacokinetics and immunogenicity (HAHA) by ELISA assay were collected in a limited number of patients post-treatment at 6 weeks (n = 12), 10 weeks (n = 7) and 18 weeks (n = 7). Epratuzumab serum levels were measurable in all available samples through at least 10 weeks post-treatment and were still detectable above the 0.5 μg/ml assay limit in 5/7 samples evaluated at 18 weeks, with median values of 120 μg/ml (range 49 to 350) at 6 weeks, 48 μg/ml (range 31 to 138) at 10 weeks, and 8.3 μg/ml (range 1.82 to 25) at 18 weeks. Figure 4 shows the individual measurements over time. There was a single sample showing 1.42 μg/ml at 32 weeks. HAHA analysis gave no evidence of immunogenicity, with all post-treatment values either remaining below the 50 ng/ml sensitivity of the assay or not increased from baseline values prior to treatment.

Immunology laboratory tests

Table 7 shows that at the first evaluation after treatment, mean B cell levels decreased by 35% and persisted at these levels on subsequent evaluations (Figure 5), with no evidence of onset of recovery by the final study evaluation at 32 weeks (6 months post-treatment). In contrast, there does not appear to be any consistent pattern of decreases/increases in T cell levels or serum levels of IgG, IgA, or IgM following treatment (Table 7).

Although all 14 patients had measurable ANA titers (1:80 to 1:5,120) at study entry, no patient had consistent post-treatment decreases, including evaluations at 32 weeks (6 months post-treatment) follow-up (8 patients had no changes at any evaluation, 5 doubled their baseline titers at one or more evaluations, and one patient had an isolated decrease at one evaluation). Five patients had elevated anti-dsDNA antibodies (10 to 123 U/ml) at study entry, but none had any decreased post-treatment values (2 patients had no significant changes, and 3 had increases at one or more evaluations). C3 levels that were decreased or borderline for 3 patients at study entry remained virtually unchanged post-treatment, as did mean C3 values for all patients.

Table 4

Number of patients with deteriorating BILAG activities from baseline

BILAG body system (N = 14)[a]	6 weeks[b]	10 weeks	18 weeks
General	0 (0 %)	0 (0 %)	0 (0 %)
Mucocutaneous	0 (0 %)	0 (0 %)	0 (0 %)
Neurological	0 (0 %)	0 (0 %)	0 (0 %)
Musculoskeletal	0 (0 %)	0 (0 %)	0 (0 %)
CV/Respiratory	0 (0 %)	0 (0 %)	1 (7 %)
Vasculitis	0 (0 %)	0 (0 %)	0 (0 %)
Renal	0 (0 %)	1 (7 %)	0 (0 %)
Hematology	1 (7 %)	1 (7 %)	2 (14%)
Overall[c]	1 (7 %)	2 (14%)	3 (21%)

[a]N = total number of patients. [b]Twenty-four hours after fourth infusion. [c]As applied to any BILAG body system.

Table 5

Number of patients in each BILAG body system with resolution of baseline B- and C-level disease activities

Body system	B level	C level
General	3/3 (100%)	0/11 (0%)
Mucocutaneous	4/13 (31%)	0/1 (0%)
Neurological	0/0	2/10 (20%)
Musculoskeletal	1/2 (50%)	1/11 (9%)
CV/Respiratory	0/2 (0%)	2/2 (100%)
Vasculitis	2/5 (40%)	0/4 (0%)
Renal	0/0	2/4 (50%)
Hematology	0/1 (0%)	0/11 (0%)

Resolution is defined as post-treatment improvement of baseline disease activity level by at least one category level (B to C, D, or E; C to D or E) at one or more evaluations up to 18 weeks, with no categorical deterioration from the baseline activity level prior to improvement, and no reversion to the baseline activity level once any improvement has occurred. Additionally note that 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks.